THREE MAJOR PATHOLOGIC PROCESSES CAUSED BY LIGHT IN THE PRIMATE RETINA: A SEARCH FOR MECHANISMS*

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INTRODUCTION

BOTH POSITIVE AND NEGATIVE EFFECTS OF THE ENVIRONMENTAL LIGHT ON THE human have been known to exist for ages. The immediate effect of sunburn of the skin could not be missed even by primitive people. A long-term effect of the sun's rays, carcinogenesis, was first suspected by association and later verified as a scientific fact.[1] The fact now supports a large present-day research effort into the mechanism by which sunlight modifies cell multiplication. The possible mechanisms range from direct deoxyribonucleic acid (DNA) damage, changing genetic code in the nucleus of the cell,[2] to suppression of the immune or cellular genetic repair system, or both, by an as yet unknown mechanism.[3] Negative biologic effects have been traced to various portions of the visible and ultraviolet spectrum, and each portion of the spectrum might be expected to produce its effect by a different combination of mechanisms. There have also been a multitude of studies of physiologic and pathologic effects of light upon the eye.

The effects upon the eye caused by exposure to various light sources are based on a varied combination of mechanisms, each of which are wavelength-, intensity-, and time-dependent. It is well known that the cornea is subject to "sunburn," which is caused by the ultraviolet wavelengths, while the visible wavelengths reach the retina and the longer wavelength infrared energy can heat the eye, particularly the iris and lens, sometimes causing lenticular changes.[4]

Damage caused by light that reaches the retina of the eye is also wavelength and time-intensity dependent. Retinal damage has been

*This work was supported in part by the following grants and contracts: US Army Medical Research and Development Command DAMD 17-80-C-D129, DAMD 17-74-C-4026, Bureau of Radiological Health #FD 00874 and Research to Prevent Blindness, Inc, Departmental Award.

caused by staring at the sun during eclipse,[5] observing an atomic fireball,[6] or intentionally by photocoagulation of the retina for medical purposes. Damage to the retina caused by light is defined as any permanent change in structure or function identifiable histologically or by other means as having its effect in the retina. The retinal damage may be cell-specific and manifested by cell loss or a reduction in the number of cells or nuclei within a retinal layer. Permanent changes also may be restricted to particular organelles of the cells and manifested by structural changes, eg, of the mitochondria of a cell. Theoretically, light damage also includes reversible pathologic changes in structure or function or both, which are repaired in several days or weeks, eg, by regrowth of the outer segments of the visual cells.

The effect of light to be discussed in this thesis is that caused by one- to four-hour exposure of the retina to the visible portion of the spectrum, at intensities less than that necessary to raise the retinal temperature 1° C.[7] The thesis presents a detailed description and evaluation of studies of various pathologic states found in the light-damaged primate retina over a ten-year period. It presents new data on the progression through these states during damage and repair. It also describes a new analytic approach to light damage, which is the study of retinal cells in tissue culture. For this, the pigment epithelium was selected and studied histologically with and without melanin pigment. Finally, the thesis presents an analysis of three separate pathways of pathologic change caused by light and relates these pathways to the exposure parameters that determine them. It presents a new hypothesis on the pathway of the damage produced by short-wavelength visible light.

The analysis of past and present work leads to the thesis that at least three effects together account for the damage sustained with the parameters given. These parameters are (1) wavelengths of visible light, (2) exposures of 30 minutes or longer, and (3) intensities within one log unit of threshold for ophthalmoscopically visible lesions for the given exposure times that do not increase the temperature of the retina significantly.

The three mechanisms to be discussed are highlighted by (1) a rhodopsin-mediated effect expressing itself in rod cell and pigment epithelium pathologic findings as described by Noell,[8] (2) a cone-type specific function effect,[9] and (3) a short-wavelength effect first described by Ham and Mueller[10] and Lawwill et al,[11] which involves inner as well as outer retinal layers. These three effects are modulated by different variables and occur together in different proportions in primate retinas and probably others as well. The isolation of any one effect is difficult to achieve in the primate retina. Each may have a distinctly different mechanism(s) of

action, but all may share similar final pathways to irreversible damage.

If isolated, the first mechanism is dependent upon visual pigment concentration in the rods. It is enhanced by long-term dark adaptation preceding the exposure. It is also enhanced by increased body (eye) temperature. The damage is first shown by the rod outer segments, but it includes ultimately the pigment epithelium and all parts of the rod cell. One might postulate a series of events beginning with the excessive but physiologic action on the visual pigment, followed by photo product-enhanced destructive oxidation or metabolic derangements or both, leading to a final common pathway of cellular degeneration.

For the second mechanism one might think of a specific class of cones being bombarded with a level of light energy in their characteristic spectral range but above normal physiologic intensity. Cone cells become "overstressed"; intracellular and extracellular changes occur that are incompatible with normal cell function and viability. Thus the cells can no longer maintain their integrity, their plasma membrane is breeched, and they die. At threshold, the majority of cells that die or are permanently changed are the cones responding to the incident wavelength.

In the third mechanism, all of the retina is bombarded with photons of a relatively high energy, bombs looking for places to explode. The photons are absorbed by many different molecules, including the melanin of the pigment epithelium, the molecules of DNA transcription and protein synthesis, specific molecules of the cell membranes, and the enzymes of important cytoplasmic or mitochondrial functions. Possibly their absorption by molecules in the nucleus causes the death of the cells. Maybe involvement of ribosomes or smooth endoplasmic reticulum or both stops the cell's synthetic machinery. Possibly also, absorption by the oxidative enzymes of the mitochondria blocks energy yield of reactions on which cell life depends. One or all of these actions may be primarily responsible for the damage caused by the short-wavelength light. This third mechanism is the prominent theme of the thesis because it seems to be the major route of acutely induced light damage in the primate retina.

BACKGROUND

ANIMAL MODELS OF LIGHT DAMAGE

The most recent surge in interest in the pathologic effects of light exposure on the retina was initiated by reports by Noell and co-workers[12] in 1966. Noell reported that irreversible retinal damage occurs in normal laboratory rats exposed continuously to an illuminated environment. He noted that the threshold for this damage was lowered when the animals'

body temperature was raised. He also noted that divided doses of light produced damage more effectively than continuous exposure of the same total length and intensity. Sensitivity to damage was greater with green light than with either red or blue light. In fact, the action spectrum of the damage paralleled the action spectrum of the electroretinogram (ERG). The iris of pigmented rats provided significant protection when compared with albino animals. Several days or weeks of dark adaptation prior to this damaging light exposure was associated with greater damage to the same light for the same period of time than in animals that had lived in cyclic light. Later Noell et al[13,14] demonstrated that vitamin A deficiency protected rats from light damage of the severe type, provided rhodopsin content was reduced by the deficiency. The conclusions drawn from this initial work were that the rod's visual pigment mediates the retina damage in the rat and that the physiologic reaction of the photoreceptor cell becomes damaged when light is strong and prolonged.

Noell divides the effects into at least two types, one expressed by the death of the rod cells and the pigment epithelium and the other by a slower, partly reversible rod cell degeneration.[15] It appears that a normal light cycle protects the rat eye somewhat from the most severe effect. Noell also notes that when the receptor cells had died and disappeared in the rdy (RCS) rat, the pigment epithelium remained unaffected by light of the same intensity and duration that destroyed it in the normal rat.[15]

Rudeen and O'Steen[16] have stressed the hormonal influence on susceptibility to retinal damage, another evidence that the normal physiologic rhythms in part determine the effect of light upon the retina. Hollyfield et al[17] discovered that continuous dark exposure could also cause receptor disorganization, but this observation was made on frogs. However, it adds another bit of interest to the hypothesis that any significant alteration in the normal rhythm of illumination can cause retinal damage or change its threshold to damage.

A more applied approach has been taken using different models. Friedman and Kuwabara,[18] Tso et al,[19] Dawson and Herron,[20] and Hochheimer et al[21] have described the retinal effects of specific ophthalmic instruments presently in clinical use. Dawson and Herron[20] began with the indirect ophthalmoscope, detecting subsequent changes in dark adaptation in patients after examination with this instrument. Friedman and Kuwabara[18] produced severe lesions with 30-minute exposures of the monkey retina to the indirect ophthalmoscope in an artificial situation where the image was held stationary. Hochheimer et al[21] produced severe retinal damage in monkeys by exposing them to an ophthalmic operating microscope for 15 minutes. These studies have given evidence

of the potential importance of light damage in ophthalmology. Lawwill et al[22] have calculated retinal exposure with the office slit lamp and Hruby lens. The possibility exists that damaged thresholds can be reached with this device.

A series of studies beginning with the effect of short atomic bomb flashes up to and including 1000-second exposures have been made by Ham et al[23] over several years. In general, they utilized small area spots and ophthalmoscopic detection of the lesions to describe the threshold under various conditions of time and light wavelength. They have published evidence that the shorter wavelength blue light (441 nm) is more effective in producing these lesions.[24]

The rabbit as studied by Lawwill et al[25] responded to light damage differently than rats, although the retinas of both species are rod-dominant. More energy was required in the rabbit, and the action spectrum was different. The rabbit retina was more sensitive to short-wavelength light than to medium-wavelength, and the pigment epithelium was relatively less affected. Damage was seen also in nonreceptor cells of the retina. These studies on the rabbit provided the basis for the extensive analysis of damage by light in the monkey's retina, the description of which forms the main part of the thesis. It demonstrates the particular sensitivity of the primate retina to short-wavelength (blue) light and the involvement of the inner as well as outer layers.

OVERVIEW OF POSSIBLE MECHANISMS

If the receptor as well as nonreceptor cells of the retina are particularly sensitive to short-wavelength light, one must postulate that the damaging light is absorbed by some molecule that is not a visual pigment (eg, rhodopsin). Since short-wavelength ocular transmission to the retina virtually cuts off at 400 nm, one may be investigating with blue light an effect several log units down from the peak sensitivity of the damaging wavelength region, or one may be at a wavelength near or at the peak. The cutoff filter of the optical media limits the transmission to the retina of the higher energy short-wavelength photons. This gives an effectiveness spectrum that is an algebraic combination of the transmission spectrum and the actual action spectrum. Thus the "effectiveness" spectrum may be very different from the action spectrum, which is required to identify the molecules that absorb the damaging light. Similar effectiveness spectrums have been well described for sunburn.[26]

There is reason to believe that at least one of the mechanisms involves the visual pigment rhodopsin, based on Noell's work showing rat retinal sensitivity peaking in the center of the visual spectrum. Whether this

effect is primarily a molecular change that is damaging or whether the bleaching of a large portion of the visual pigment sensitizes the cells metabolically to damage or releases a chromophore to mediate a photosensitive reaction is not known. There is also evidence from Harwerth and Sperling's work[9] that repeated exposure to low-intensity light may damage cone cells at wavelengths that peak at the absorption maximum of the specific cone pigment. This evidence will be further discussed in the main body of the thesis.

An area not covered by this thesis is damage by heat. An important distinction must be drawn between heat and photobiologic effects. It is a distinction between molecular rotational and vibrational effects v intramolecular events. Absorption spectra and chemical bonds are peculiar to a particular molecule. A photon may be absorbed only when its energy matches the energy required for a possible transition between energy states of the molecule. The energy supplied by a photon is determined by its wavelength ($E = hn$). The critical energies for photobiologic reactions straddle the visible spectrum. On the one side, UV photons are quite effective in causing transition; on the other side, infrared has no significant photochemical action. When a transition is caused by the absorption of the photon, the change in electron orbital position may cause formation or breakage of a chemical bond. This would be a photobiologic effect as opposed to the generalized effect of molecular motion expressed as a rise in temperature. Raising the basic molecular kinetic energy, heating, may enhance superimposed photobiologic reactions. In general, this thesis addresses a complex interaction that includes photobiologic as well as physiologic metabolic processes, in which there are many energy transitions, not necessarily primarily photic even though the first step is light-dependent.

When the energy state of a molecule has been raised, it seeks its basal level. Return can be achieved by emitting another photon, called fluorescence. A state of molecular excitation may be a singlet state with normal electron spin, a short-lived state of high reactivity, or it can transform into a triplet state with reversal of electron spin. The triplet state can last several seconds and is also highly chemically reactive. Sensitization to photobiologic reaction can occur when the sensitizing agent by chemical binding either increases the absorption of photons of a particular wavelength or decreases the activation energy required for a reaction. Photochemical reactions that are biologically damaging are often through intermediates such as singlet oxygen, peroxides, and other radicals or excited molecules. In the retina, it would be most useful to know what possible sensitizing compounds or molecules are normally present or

might be produced by light exposure. Cell components that may be altered directly by these reactions include nuclear DNA, protoplasmic RNA and proteins, and the membranes of cellular organelles and of the cell membrane. Most studies suggest DNA effects are most prominent with high-energy photons of wavelengths less than 300 nm. Dimer formation, strand breakage, and cross-linking are possible effects that may permanently alter the cell. Damaging effects on enzyme systems could be acutely devastating to the cell. Energy-yielding processes carried out in the mitochondria are of particular importance, as will be discussed in the thesis. Membrane damage by visible light has been reported in microorganisms.[27]

OVERVIEW OF ANATOMIC AND FUNCTIONAL CHARACTERISTICS

The primate retinal layers are made up of cells with vastly different functions, which are in the most part highly specialized. Not only are the cells specialized but so are the areas of the retina, ie, macular, peripheral. Beginning with the outer layers, the choroid is important as a supplier of nutrients and a heat sink and energy absorber. The heat sink function is not as important in low-level light damage, but the nutrient function may be. A secondary effect of a long light exposure is edema and cellular swelling and disruption of the function of the pigment epithelium. The receptor cells may become physically separated from the pigment epithelium, disturbing the metabolic support of the receptors. Gross separation[28] as in retinal detachment is known to cause degeneration of receptors, and minor separation may play a part in the damage attendant with light exposure.

Bruch's membrane has been found thickened in light damage.[11] The membrane of the pigment epithelial cells apposed to Bruch's membrane has a peculiar enfolding that is suceptible to damage. The pigment epithelial cells are pluripotential and may form phagocytic cells after the intercellular tight junctions are broken. The apical membrane of the pigment epithelial cells carries the microvilli. These extend up between the outer segments to meet the Müller cell processes coming the other way. At the base of the microvilli are the cigar-shaped melanin granules palisaded all in a row at the inner surface of the cell. This is an area of concentrated light absorption, a part of a very carefully arranged black screen. The granules also may have physiologic or chemical properties of absorbing toxic radicals[46] along with their function of absorbing light. In light damage this is one of the first or a prominent area of structural change.[11]

The outer segments are fragile structures containing a stack of membrane discs with an exceptional phospholipid matrix of high fluidity. They

are continually renewed by new membrane formation at their base, where visual pigment incorporation occurs. Light damage is frequently associated with a disorganization of outer segment membranes. The inner segments, on the other hand, are packed with mitochondria reflecting the high metabolic needs of the cells. The mitochondria of the rods are long and slender compared with the thicker ones in the cones. Specific functional differentiation might be suspected but is not known. Light damage, as will be described, affects differentially the mitochondria of rods and cones.

The outer nuclear layer contains the nuclei of the rods and cones with the cone cells lining the outer edge of the layer with their more lightly stained nuclei near their wide inner segments. Sometimes one or two cone nuclei are even found in the inner segment. Cells in the outer nuclear layer show damage by pyknosis or karyorrhexis as well as karyolysis and are lost individually in light damage.[11] There is no reason to expect strong absorbing properties of this layer to visible lights as one does for the outer segment with its visual pigment and the inner segment with its stacks of mitochondria and concentrated cytochromes and flavins.

The outer plexiform layer contains the proximal processes of rods and cones making contact with the processes of the next neurons. This layer is particularly susceptible to edema or swelling of cellular processes in light damage.[11] The biopolar cell layer has several specialized types of cells. This layer has the most distal extension of the capillaries, while the choroid is the source for the nutrients to all outer layers. Therefore the level of nutrients and oxygen tension may vary significantly throughout the receptor cell regions. This is important because many photochemical reactions that are biologically harmful are oxidative and depend upon the concentration of oxygen. Formation of singlet oxygen, which is highly reactive, is believed to be the first step in a cascade of reactions that lead to the destruction or inactivation of a biologic system during light exposure.[47]

The ganglion cell and nerve fiber layers are nearly transparent, but the interface with the vitreous is prominently seen with deep blue illumination. Fiber layer defects in glaucomatous eyes can be seen in blue light with the ophthalmoscope. The latter suggests that blue light transmission is affected at this border by scatter and reflection.

A particular kind of specialization in the retina is represented by the fovea and macula. In the center of the macula, the most proximal retinal region is the outer plexiform layer, and just lateral to this the inner layers are usually thickened. A yellow carotenoid pigment derived from the diet is accumulated[29] in this region and is most concentrated in the center 1

mm, decreasing rapidly over a 1- to 2-mm radius from the center of the fovea. This yellow pigment layer partially screens the area from short-wavelength light. It has an optical density of approximately 0.5 at 460 nm, 0.2 at 515 nm, and 0.1 at 590 nm.[29] Other specializations of interest in the macular area include a high concentration of slender-type cones and almost total exclusion of rods. Between 10° and 15° from the fovea the highest concentration of rods exists.[30] The melanin pigment concentration in the pigment epithelium also shows a gradient with the highest concentration in the central macular area, decreasing toward the periphery. All these differentiations may have a part in determining the type and distribution of damage by light. There may also be metabolic differences with similar circumferential distribution around the fovea.[31]

In the rabbit these differentiations are arranged linearly instead of circumferentially. More of the inner retina derives its nutrition from the choroidal blood supply. The area of the most acute vision in the rabbit lies in a horizontal strip along the visual streak anatomically defined. There is no specialized accumulation of cone cells in this region. In spite of these differences, light damage in the rabbit has many similar features, as found in the monkey.[25]

The retina is metabolically an extremely active organ, and a great portion of this activity is located in the photoreceptors, especially in the absence of light stimulation. The neural cells are limited in their biologic activity, while the pigment epithelial cells perform many functions and can dedifferentiate and respecialize in some of these functions, most notably phagocytosis. In addition, they may have the ability to divide. All retinal cells are liable to denaturing events, ie, thermal covalent bond-breaking and high-energy photon-induced enzyme or genetic defects. The reparative powers of the retinal cells are probably very limited.

OVERVIEW OF PHYSICAL CHARACTERISTICS

In order for light energy to cause pathologic changes, it must be absorbed. The portion of energy absorbed in any tissue is dependent upon the transparency or opacity of the tissue for the incident light. The transparency is dependent upon the wavelength components of the light. Just as a red filter passes longer wavelength light and absorbs the shorter wavelength blue and green light, the cornea absorbs essentially all of the ultraviolet (UV) below 310 nm.[32] Most of the near UV light between 310 and 400 nm is absorbed in the lens.[32] Thus, one would look first in the cornea for ocular damage from short wavelengths and in the lens for longer ultraviolet wavelength (UVA) damage. If not all of the near UV light were absorbed in the cornea and lens but passed further into the

eye, and other components were sensitive, one might find damage deeper in the eye.

Transmission of the short-wavelength light to the retina is increased in the aphakic condition. The ocular spectral transmission curve and the transmission curve for each component of the media and cellular layers is of utmost importance in knowing where in the eye each portion of energy at each wavelength might be absorbed. Several curves have been ascertained by direct measurement and by calculation.[32] Since each eye differs and marked changes also occur with age, only a general characterization is valid. The shortest wavelengths are stopped at the corneal epithelium and at high intensity can cause damage there. The arc welders' superficial keratitis and the "eyes-open sun lamp enthusiasts' keratitis" are the typical human examples. The sharp cutoff "filter" of ocular transmission to the retina for short wavelengths is at about 400 nm and is attributable to absorption in the crystalline lens. The transmission changes several log units within a few nanometers. This does not prevent a very small fraction of the energy at wavelengths shorter than 400 nm from reaching the retina, but more posterior elements would have to be uniquely susceptible to sustain damage from wavelengths shorter than 390 nm. However, in the aphakic eye a significantly larger portion of the energy with a wavelength longer than 310 nm is transmitted to the retina. With the lens in place, cutoff at the red end of the spectrum is not as sharp but definitely exists. Heating of the front of the eye occurs in glass blowers, causing effects in the crystalline lens without obvious effects on the retina. Almost any type of radiation in reasonable quantity can affect the crystalline lens in situ, causing temporary or permanent opacification. The extremely long wavelengths of the CO_2 laser are effectively stopped at the ocular surface. Damage to the tissue outside of heating effects, which require a great deal of energy, have not been shown to be prominent at these longer wavelengths. Therefore, for the purposes of this presentation, the cutoff at the longer wavelengths is of less importance. One should note that between 400 and 700 nm, the spectral transmission curve of the normal human eye is nearly flat and percentage transmission to the retina very high.[32] The problem for the future is this: What portion of the energy at each wavelength between 380 and 700 nm is absorbed by which molecules in each retinal layer and by each retinal cell or structure?

The light that damages must be rigorously qualified and measured in its quantities. Many units have been used to measure light incident upon the retina in the light damage literature. Much confusion can arise from inexact or inaccurate measurements. Comparison of studies is sometimes difficult when spectral intensity curves of sources are not provided. In

general, measurements are made in physical units relatable to intensity and wavelength or in physiologic units related to the production of the visual response, photometric units. Physical units are watts for power or intensity and joules for energy or power times time. These are then related to area, cm^2, or solid angle, steradian, and may be broken down per nanometer of wavelength.

Photometric units represent a potpourri of colorful names with difficult relatability to the physical units, ie, nits, apostilbs, lux, lumens, trolands, candelas, and others. All have their origin and utility in psychophysical studies, where detectability and perceived brightness are the important factors. These measures have no place in the quantitative study of retinal light damage.

If light damage were solely a function of visual pigment bleaching, photometric units would be appropriate. But, if one starts by using photometric units, the measures of true importance may be obscured because the visual pigment may not be involved and must be factored out again. As well, the human visual spectral response on which these units are based is not applicable to other animals. In the case of short-wavelength light damage, a blue light (457 nm) that is of equal intensity (watts per cm^2) to a green light (514 nm) is not only more damaging in the monkey but also less bright (has lower photometric value). Thus, the best record of light falling on the retina is in watts per cm^2 with a spectral distribution curve supplied.

This thesis covers effects of light when applied to the retina over an extended area for an extended time. It is known that the dynamics of damage can change with changes in the size of the area of exposure.[33] This is particularly true of damage related to heating that may be dependent upon the qualities of the surrounding retina as a heat sink. The local conduction of heat becomes important for exposures lasting longer than a fraction of a second, and adjacent blood flow in the choroid becomes important for longer exposures.[33,34] The problems of light damage dependent upon instantaneous or prolonged heating of tissues or instantaneous heating by a pulse of energy are not a theme for this thesis. Also excluded are the mechanical effects of the shock wave associated with the ultrashort pulses of q-switched lasers.

EVALUATION OF LIGHT DAMAGE

Damage can be described anatomically or functionally. The study of functional effects helps in understanding the sequence of events that occur in retinal light damage and that may not be revealed by histologic findings. Both temporary (reversible) and permanent (irreversible) dam-

age occur, and some damage may be detected on the functional level only. Sailors who stare over open water show measurable delay in dark-adaptation and raised thresholds,[35] as do persons who are examined by indirect ophthalmoscopy.[20] The histologic status in these type of conditions has not been determined, except for direct sungazing.[36]

Visual function tests have been put to particularly good use by Sperling and co-workers in their monkey studies.[9,37] They have shown color-specific destructive light adaptation in primates using light levels approximately three log units below those for the short-wavelength light effect in the monkey experiments to be described. These effects are most convincingly shown for the "blue" cone receptors, but are also reported for the medium-wavelength receptors.

The flash ERG is a well-known measure of retinal receptors and bipolar layer function. It reflects adaptational and luminance response in a highly predictable and repeatable way.[38] It can be used to determine the gross state of function of rods or cones, and can detect gross changes in more inner layers. The a wave reflects the outer segment response, while the b wave is dependent upon activity in the bipolar cell layer. The bar-pattern ERG, once thought to reflect these same layers, is now believed to also reflect activity in the inner plexiform and ganglion cell layers. Both techniques combined are particularly appropriate for the study of light damage in the retina. The visual evoked response (VER) is recorded from the scalp or skull and provides a record of the integrated response for the retina. It can be used to measure visual function for the aspect of resolution. It is particularly important in evaluating lesions in the macular area, since it is representative mainly of the response from the central 5° of the visual field.

The most important techniques for evaluating permanent damage are the anatomic ones of light and electron microscopy. With respect to the short-wavelength damage, they were first put to use by Lawwill et al.[39] They demonstrated that light damage is much more complex pathologically than originally believed from work on the rat.

Figure 1 demonstrates the value of histology in describing and grading pathologic changes from light damage. These five panels are drawn from the rabbit model. They depict the normal retina in section 1 and progressively more severe damage in sections 2 through 5. The lowest levels of detectable damage are shown in Fig 1 A, bottom. In the pigment epithelium, the plentiful melanin pigment is normally located. The outer segments are shortened and distorted. The inner segment mitochondrial area shows many small vacuoles and general cell swelling. A halo is around the nuclei in the outer nuclear layer. In Fig 1 B, top, the pigment

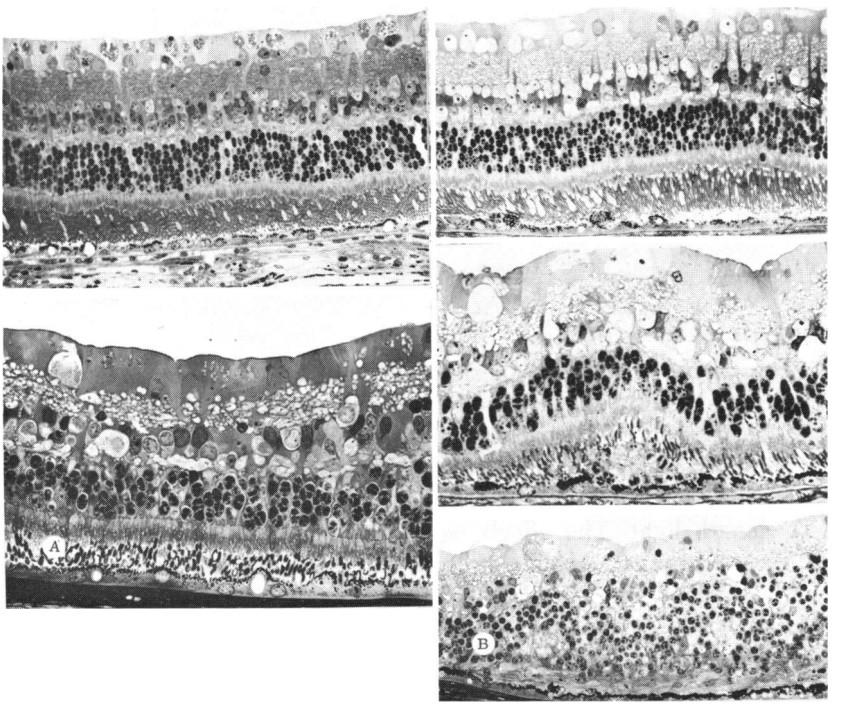


FIGURE 1

Series of five rabbit retinas from normal to extreme light damage. A: Upper panel is normal control (toluidine blue, ×150). Lower panel shows 1.5+ damage with swollen and shortened outer segments. Inner segments have lucent areas that would correspond to swollen mitochondria seen with electron microscopy. Cells in outer nuclear layer have nuclear halos. There are pyknotic cells in ganglion cell layer (toluidine blue, ×376). B: Upper panel represents "2.5+" damage. Pigment epithelium is thinned; phagocytic cells contain much pigment. Pyknosis and ghosting are seen in inner layer (toluidine blue, ×150). Two lower panels show greater (3+ and 4+) damage (middle panel, toluidine blue, ×375; lower panel, toluidine blue, ×375). All layers are finally lost except outer nuclei and thin pigment epithelium.

epithelium is distorted and has lost some of its melanin to macrophages in the subretinal space. The outer segments are distorted and their ends are swollen. Some may be missing. There is thinning of the outer nuclear layer and swelling of both plexiform layers, the inner nuclear layer, and the ganglion cells. This is moderate damage, graded 2+ on a scale from 0 to 4. More severe damage (3+) is shown in Fig 1 B, middle. Here the pigment epithelium appears almost necrotic. Melanin distribution is abnormal. Outer segments are completely disorganized. Inner segments are grossly swollen and some are missing. The outer nuclear layer is thinner. The bipolar and ganglion cells are necrotic and all their processes are

enlarged. In the last section, Fig 1 B, bottom, severe (4+) damage is shown. All that remains are dislocated receptor nuclei, the damaged pigment epithelium, and a few cells of the inner layers.

These findings and the levels of light required to produce them contrast with previous findings on the rat and lead to the necessity for a primate model, which is extensively discussed in the following sections of the thesis.

EXPERIMENTAL RESEARCH

GENERAL INTRODUCTION

To answer the many open questions on light damage and its mechanisms, a large-scale study was launched in my laboratory to analyze light damage produced by long-term exposures in the monkey. All physical variables of light exposure were rigidly controlled and accurately quantified. The exposures covered a large area of the fundus with an even field of various wavelength light. The effects were carefully measured by an array of clinical, functional, and anatomic examinations. Functional measures included flash and bar-pattern electroretinography and the recording of the visual evoked cortical response. Clinical examination included indirect ophthalmoscopy and fluorescein angiography. The anatomic examinations included examination by light and electron microscopy.

The monkey was chosen as an experimental animal because of its relevance to patients, being the closest possible model to the human. A four-hour exposure period was initially selected to be short enough to achieve accurate dosimetry and long enough to simulate the environment to which patients might be exposed.

Later, in order to prepare for the study of any cumulative effect that might be present from multiple repeated exposures, and to describe any time-interval dependence, thresholds for one-hour exposures were established. It was of practical importance to know whether light damage found from a divided dose regimen would have additive, less than additive, or potentiating effects. This portion of the project was also undertaken to analyze mechanisms dependent upon visual pigment concentration or the metabolic state of the retina or both. If short exposure times produced their damage through the visual pigment or shortlived photoproducts, one should expect a pathologic finding similar to the rat model in Noell's damage of the so-called first kind.

Also, it would be convenient for technical reasons to use a shorter period of exposure than four hours if the pathologic changes produced were the same. This was the case, and therefore one-hour exposures were

chosen to follow the progress of damage in the latter part of this experiment.

The studies were performed on more than 100 monkey eyes followed up to eight months after exposure. They yielded unambiguous results, permitting a virtually complete description of the phenomenology of light damage in the primate.

In the following, three separate experiments will be presented with their methods, results, and discussion in sequence. Experiments 1 and 2 present the work on monkeys. Experiment 3 is an approach to studying light damage in tissue culture of the pigment epithelium.

EXPERIMENT 1: LIGHT DAMAGE IN THE MONKEY RETINA

In the description of experiment 1, I will give an account of data collected with 80 monkeys on the damaging effects of exposure to laser lights with emphasis on the cellular changes revealed by light and electronmicroscopic observations.

METHODS

Animal Preparation.—Female rhesus or cynomolgus monkeys weighing between 3 and 4 kg were used. Prior to exposure, the animal was given 0.12 mg of atropine sulfate and 8 mg of phencyclidine hydrochloride (Sernylan) intramuscularly (IM). When the monkey was sedate, it was placed in a primate chair. Initiation of anesthesia was by means of a single 20 mg/kg intravenous dose of sodium pentobarbital. A constant intravenous infusion of sodium pentobarbital (0.22 mg/min, 2.84 mg/ml) was begun a short time later and continued throughout the exposure. The eye to be exposed was dilated with one drop each of atropine 1%, tropicamide 1%, and phenylephrine 10%.

Light Exposure.—The light was presented to the eye in Maxwellian view. The eyelid was held open by a Burian-Allen contact lens electrode. This protected the cornea and kept it moist. The alignment was maintained by the experimenter's sighting through a beam splitter to allow direct visualization of the exposed fundus. The intensity of exposure was constantly monitored via a beam splitter in the light path reflecting on an Eppley thermopile. Exposures were for a period of from 15 minutes to 4 hours with a constant-intensity light source covering 40° solid angle (0.88 cm^2 of retina). The intensity of the beam was measured with a spectroradiometer (Gamma Scientific model 2020a) calibrated with a standard of spectral irradiance traceable to the National Bureau of Standards. The homogeneity of the field was determined with a small (2.5-mm diameter) cosine receptor by measuring the intensity at the center and edge of the field. Uniformity was maintained within 50%.

The retinal area exposed was calculated both from the angle of convergence of the incident beam and from direct measurement of the chord of the exposed section and the diameter of the eye using a freshly enucleated monkey eye. The area value used in calculating the irradiance was taken from the direct measurement. There was about 20% difference between the two results.

Electroretinogram Recordings.—The ERG in response to a brief and intense flash was recorded binocularly prior to exposure at least twice a week until a stable pattern was achieved. After exposure, recordings were made at 24, 72, and 144 hours after the end of exposure, and two or three times per week thereafter. In brief, the animal was tranquilized, following pretreatment with atropine sulfate, IM (0.012 mg/kg), with 8 mg phencyclidine hydrochloride, IM, and the pupils were dilated with tropicamide 1% and phenylephrine 10%. Modified Burian-Allen monkey contact lens electrodes were inserted. The animal was preadapted in 1370 candelas/m^2 white ganzfeld hemisphere. The flash stimulus was provided by a photostimulator (Grass PS2) with the intensity set at 16. The flash lamp of the Grass instrument was placed inside the hemisphere to provide a ganzfeld-type stimulus. Each recording session began in the dark three minutes after completion of the two-minute period of light adaptation. Records were taken every 3 minutes for 27 minutes. Evaluation of the functional damage after exposure was made on the basis of the decrease in amplitudes of the a and b waves and the persistence of this decrease after exposure. The response of the opposite eye served as a control. The damage was graded on a scale from 0 to 4+.

Clinical Procedures.—Ophthalmoscopic examination and fundus photography were performed regularly before and after exposure with the indirect ophthalmoscope and Zeiss fundus camera. A change was graded on a scale of 0 to 4+, without the grader having knowledge of the exposure level. The slightest question of edema or pigmentary change was graded as ±. A definite change in appearance of the fundus, no matter how transient, was graded 1+. A 4+ grading was assigned when there was extensive damage of retina and pigment epithelium. Fluorescein angiography was performed prior to exposure on each eye and again after exposure.

Tissue Preparation for Histologic Evaluation.—Following enucleation the globe was suspended by the four rectus muscles in a dish and covered by a solution of 3% glutaraldehyde in a 0.1 M phosphate buffer. The cornea was removed with a trephine, and four radial cuts were made through the sclera between the rectus muscles. The zonules were cut and the lens removed. A syringe was used to gently flush the eye with fixative,

and the vitreous was cut out with scissors as it was forced through the anterior chamber opening. The whole procedure was completed less than three minutes after enucleation. These eyes were then left in fixative for 24 hours at room temperature.

After fixation the eye was placed in phosphate buffer and washed to remove the glutaraldehyde. Radial cuts were made passing through the macula to divide the globe into octants. These eight pieces of retina were placed into tubes and labeled accordingly. The tissue was washed three times for 15 minutes in buffer, postfixed for 1 hour in 1% osmium tetroxide at 4° C, dehydrated with an alcohol series, and then infiltrated with a plastic embedding media. Each octant was then bisected radially to divide the globe into 16 pieces. Each of these pieces was cut into four parts with cuts running perpendicular to the bisecting one. Thus 64 pieces of retina were obtained. They were oriented in flat embedding molds and were hardened overnight at 65° C. Sections 1 μ thick were cut from selected blocks on an ultramicrotome, placed on glass microscope slides, and stained with a 1% toluidine blue-O in a phosphate buffer. After rinsing and drying, a cover slip was applied and the section was viewed under oil on a Zeiss photomicroscope. Structures could be viewed that were not seen with the conventional hematoxylin-eosin-stained, paraffin-embedded tissue. Selected blocks were then thin-sectioned triple stained with lead citrate, uranyl acetate, and Reynold's lead citrate and processed for electron microscopic examination.

Histologic Evaluation.—After viewing several thousand sections of normal and light damaged monkey retina, it was found that the same descriptive terms were continuously used. Some terms were peculiar to specific retinal cell types or even to specific intensity levels of exposure. These realizations led to the formulation of a classificatory rating system in which clusters of descriptive terms were placed on an ordinal scale of five steps ranging from 0 to 4. Midstep ratings expanded the scale to nine steps.

Figure 2 is a reproduction of the scoring sheet that was completed for each retinal section. The terms appearing in column 3 were used when minor histologic changes were noted. The assignment of a 0.5 or 1.0 rating was dependent upon the degree or incidence of the effect observed. It was not unusual that a rating of 0.5 was assigned to nonexposed retinas. Very rarely, however, was a 1.0 rating given to an untreated section. Therefore, damage required grades greater than 1.0. While this grading is a somewhat subjective system of evaluation, it proved to be internally consistent and reliable on both intraobserver and interobserver tests.

Spec.#		Grid #			Animal #		
		Code	0-1	2	3	4	Remarks
1	PE		o vacuolization o edema o hyperchromasia o ghosting	o phagocytes o pigment shift o ↑ in lysosomes	o ↑ pigment o pigment balling o absence of pigment	o absence o ↑ phagocytes o hyperplasia o dead cells	
2	OS		o disorientation o slight swelling	o phagocytes o mild swelling	o extreme swelling o bizarre forms	o absence o ↑ phagocytes o dead cells	
3	IS		R C o o vacuolization o o edema	R C o o phagocytes o o slight swelling	R C o o extreme swelling	R C o o absence o o ↑ phagocytes o o dead cells	
4	ONL		R C o o vacuolization o o edema o o hyperchromasia	R C o o phagocytes o o ghosting o o nuc.halo o o chrom.clumping	R C o o lg.nuc.halo o o ↓ cell numbers	R C o o absence o o ↑ phagocytes o o pycnosis o o dead cells	
5	OPL		o vacuolization o edema	o phagocytes o slight swelling	o extreme swelling o ↓ cell numbers	o ↑ phagocytes o dead cells	
6	INL		o vacuolization o edema o hyperchromasia o ghosting	o phagocytes o nuc.halo o chrom.clumping	o lg.nuc.halo o ↓ cell numbers	o absence o ↑ phagocytes o pycnosis o dead cells	
7	IPL		o vacuolization o edema	o phagocytes o slight swelling	o extreme swelling	o absence o ↑ phagocytes o dead cells	
8	GCL		o vacuolization o edema o hyperchromasia	o phagocytes o ghosting o chrom.clumping o slight swelling	o extreme swelling	o ↑ phagocytes o pycnosis o dead cells	
9	Chor.		o edema, slight o ↑ inflammatory cells o ↑ size Bruchs Membrane		o vessel occlusion o ↑↑ inflammatory cells o breaks in Bruchs Membrane		
10	Ves.		o thickening of walls o ↑ inflammatory cells		o occlusion		

The rating scale is ordinal. Therefore, one can say only that a grade of 2 represents a greater effect than a grade of 1. Similarly, the same can be said of a grade of 1.5 compared with 1.0. However, the difference between grades 1.0 and 2.0 does not represent the same increase in effect as that by the step from 3.0 to 4.0. For this reason, only ordinal statistics are appropriate for analysis. If one treats the expanded scale (nine steps) as ranks with respect to damage, then one can compute the median rank observed for the retinal area exposed at a particular irradiance. The function relating median rank and exposure is a dose-response plot, and it assumes the familiar ogival form. Since grades greater than 1.0 are a consequence of light exposure, the lowest irradiance yielding a median grade greater than 1.0 (ie, 1.5) is taken to be the exposure threshold for a particular wavelength and cell type. Then, a plot of the threshold irradiance as a function of wavelength yields the action spectrum of damage.

RESULTS

Electroretinographic Changes.—The flash ERG over the course of dark adaptation was measured in over 100 eyes before and after exposure. Exposures intense enough to produce a functional deficit yielded an equal reduction in the a and the b wave, which never exceeded 50%. The time course of dark adaptation as measured by the ERG was not affected. The degree of deficit, as determined by the reduction in ERG amplitude and the period of time over which this reduction occurred, was correlated with the exposure intensity. At three days after exposure the greatest reduction in amplitude occurred, being less at shorter postexposure intervals. In most cases, with the exception of the most severely damaged eyes, significant recovery in the ERG was observed. Figure 3 shows that this recovery may be complete and that the amplitude of the ERG of the exposed eye may return to the control level.

It is important to realize that the exposed retinal area was limited to 40° or 12% of the whole retinal surface. Thus the recorded ERG was mainly determined by the function of the nonexposed retina. Maximally only a fraction of the ERG could be abolished, depending upon the loss of cells

FIGURE 2

Score sheet for evaluation of histologic findings. Each anatomic layer from choroid to ganglion cell layers is evaluated with respect to presence or absence of features entered in row corresponding to layer under study. If observed features fall into only one column for a particular layer, then that layer is given damage code appearing at head of column. If features are noted that occur in adjacent columns, damage code would be average of two column headings.

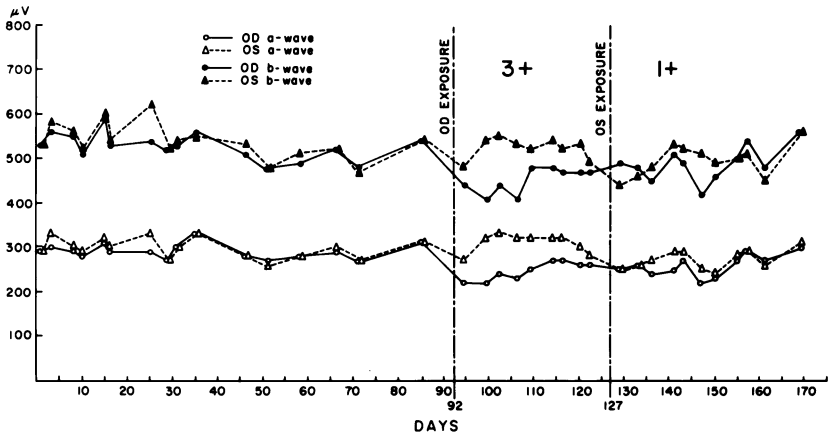


FIGURE 3

Electroretinogram (ERG) (a and b waves) before (day 0 to 93) and after exposure of right eye at day 92, and the left eye at day 127, macaque. Each ERG was measured after 21 minutes of dark adaptation. Day-to-day variation in response amplitude before exposure averaged 7%. Following exposure of right eye to 514.5 nm for four hours at 73 mW/cm^2, a 20% and 30% reduction in a and b wave amplitudes, respectively, were measured. Reduction was greatest from three to ten days following exposure. By three weeks, ERG amplitude had almost recovered to levels recorded in nonexposed left eye (OS). Minimal changes were produced in ERG of OS following exposure to 514.5 nm for four hours at 30 mW/cm^2.

in the exposed region and the "shunting" of the ERG generated by the nonexposed region through the damaged area.

Clinical Measures.—Indirect ophthalmoscopic examination of exposed eyes revealed that the earliest evidence of pathologic change was edema, which coincided with the damaged area later seen in histologic evaluation. Opacification peaked at 72 hours followed by return of transparency in approximately five days. This loss of transparency of the retina was interpreted as edema. When the edema subsided, it was followed by mottling of the retinal epithelium, which is heavily pigmented in the monkey. In severe damage, irregularity in the retinal surface was noted occasionally. No changes were observed in the optic disc or retinal vessels. A photograph of a normal fundus is shown in Fig 4 A. The same fundus after a 457.9-nm exposure is shown in Fig 4 B. One can see the ring of depigmentation, which is at the outer edge of the macula, as well as some decrease in pigmentation extending out toward the temporal vessels. This area first appeared edematous and later mottled or depigmented. The edema peaked about 72 hours after exposure.

Fluorescein angiography showed lucency of the retinal epithelium and baring of the choroid ranging from window defects just at the edge of the

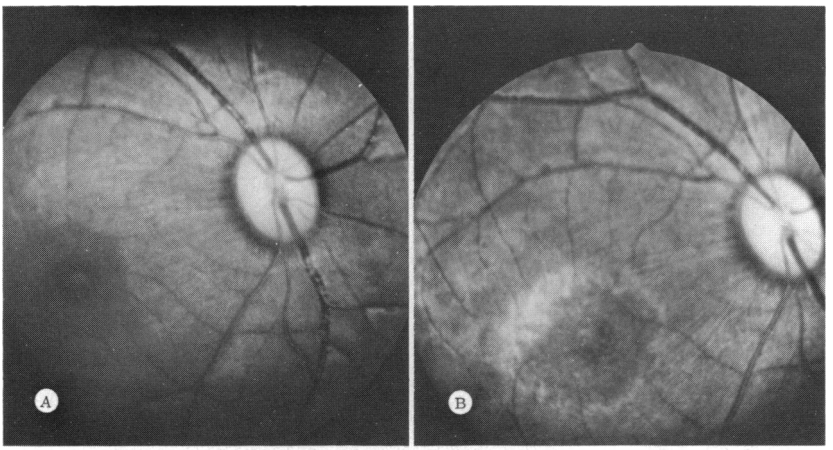


FIGURE 4

A: Normal fundus of cynomolgus monkey prior to exposure. B: Same fundus several weeks after exposure to 457.9-nm light for four hours at intensity twice average threshold. Macular area is lined by ring of depigmentation, at about 3° to 5° from center. Depigmented area has histologically the most significant damage. There is some mottling of pigment epithelium within macular area. Pigmentation is decreased all the way out to temporal vessels, although this is difficult to determine in this black and white photograph.

macula to complete transparency of the retina out to the temporal vessels depending upon the degree of damage. The defect persisted as long as any animals were followed. There was little evidence of discrete fluorescein leakage, but extensive late staining or persistent choroidal blush was usual. This occurred in the area of damage and was probably related to the loss of pigment epithelial integrity. Retinal vasculature was essentially not affected except for some pooling of dye around the larger vessels in late photographs. These findings are demonstrated in Fig 5 A and B, which are postexposure early and late phase angiograms.

Histologic Changes (Exposures at Various Wavelengths and for Various Durations).—Typical examples of light damage in the layers of the retina will be presented here by photomicrographs of both light and electron microscopic sections. The sections are selected for the pathologic changes they demonstrate. The degree of damage in any section may not correlate with the level of exposure for that eye because almost any level of damage may be found somewhere in a severely damaged eye. The level of overall light damage for the eye is classified by the most severe damage found in any area, although a damage grade is recorded for each layer in each section and for each section as a whole.

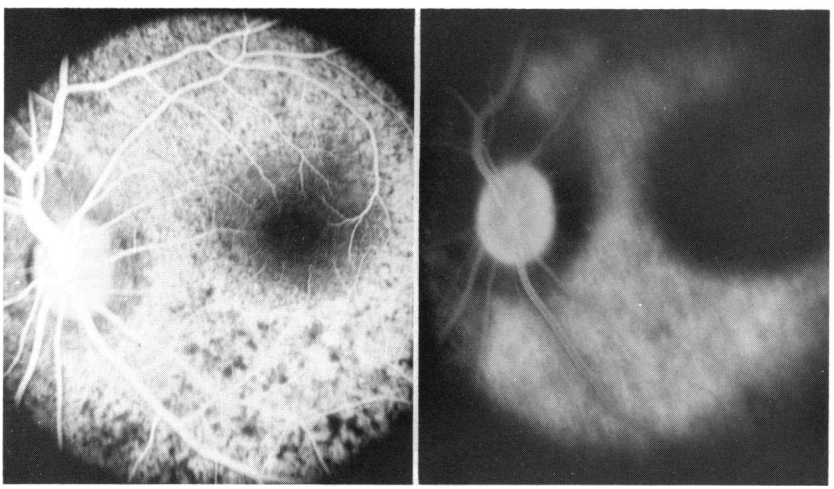


FIGURE 5

A: Early-phase fluorescein angiogram taken several weeks after exposure to 457.9-nm light for four hours at level 0.5 log units above threshold. Overall histologic grade for this eye was 4+. It shows increased lucency of pigment epithelium more prominent in area between 3° and 7° eccentric from fovea and inferiorly. There is no evidence of leakage from major vessels. B: Late-phase fluorescein photograph of same eye showing extensive staining in distribution of light damage. There are two potential sources for this apparent late staining: (1) choroid, which normally leaks fluorescein from its vessels, has been source of fluorescein that has come through damaged Bruch's membrane and pigment epithelium to lie in retina, and (2) this rather diffuse staining is normal staining of choroid seen through depigmented retina. Other fluorescein angiograms have shown patterns modified by retinal vessels suggesting that at least part of staining is in retina proper.

Light Microscopy.—The most striking changes seen in severe light damage are changes in the pigment epithelial and outer segment region. This is particularly noted in sections taken several months following exposure. The changes in pigmentation as seen grossly by viewing the eye cup are illustrated in Fig 6.

In the photomicrograph of Fig 7, the pigment epithelium is denuded and the outer segments are missing. The exposure here was intense, approximately 0.3 log units (two times) above threshold for the 457.9-nm, one-hour exposure. There are phagocytic cells in the subretinal space and severe swelling of the remaining cone inner segments. Nuclei of the rod and cone cells show pyknosis, while cell processes in the outer plexiform layer are swollen. Although the major destruction extends from Bruch's membrane to the middle of the retina, some swelling and cell loss is evident in the inner layers. Damaged areas such as this occur in a patchy but localized distribution in a zone beginning 1° or 2° from the fovea and

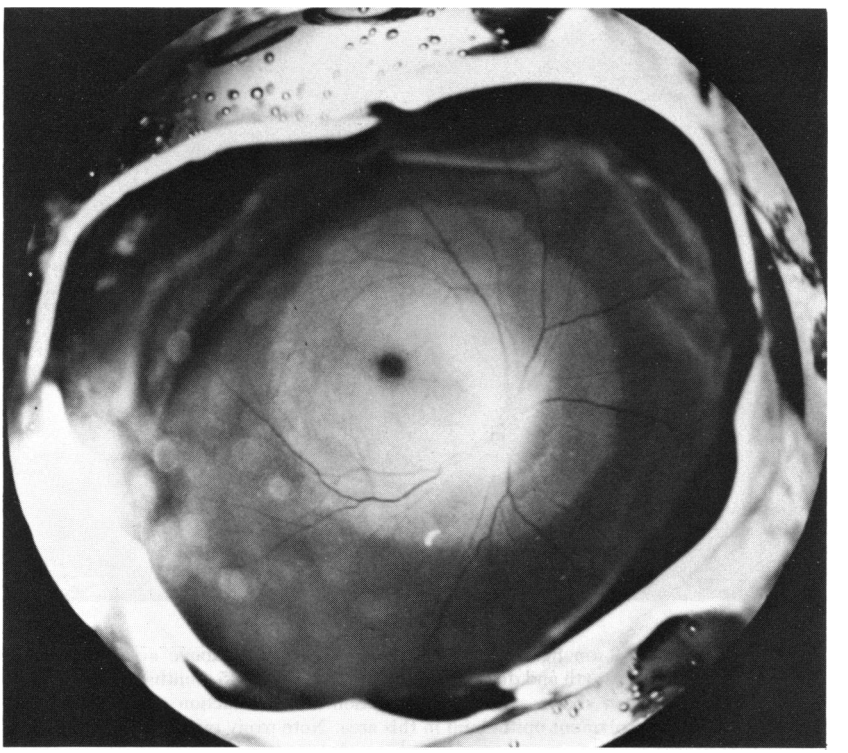


FIGURE 6
Gross photograph of fundus of exposed eye with anterior segment removed. In this case, exposure was extremely intense and affected pale area covers exposed retina. Lighter appearance is secondary to loss of pigment in pigment epithelium. This eye was exposed to 457.9 nm for four hours at intensity four times average threshold. Overall damage grade was 4+.

extending concentrically out, depending on the exposure intensity. They rarely extend beyond the temporal vessels.

In eyes with the illustrated severe degree of damage, damaged areas may be immediately adjacent to areas showing little or no change. Figure 8 is such a section from the same eye as in Fig 7, only slightly more eccentric. It shows preservation of pigment epithelium, even fairly normal location of the pigment in spite of overlying macrophages. The rod outer segments are fairly well intact although distorted. Cone inner segments show swelling. Some cone nuclei are fading and many rod nuclei are hyperchromatic. Condensed cytoplasm in the processes in the outer plexiform layer is noted. The two panels shown in Fig 9 are from the same tissue block and are adjacent except that one panel of equal width is

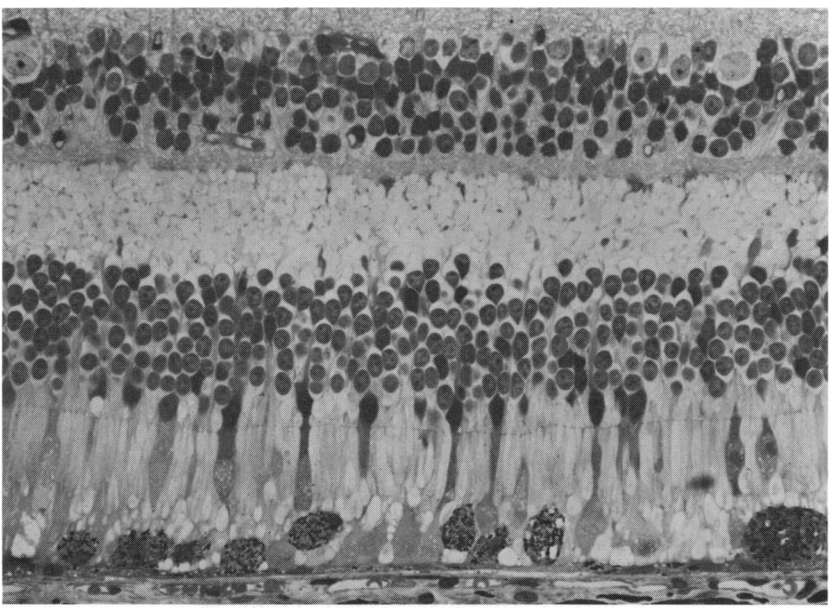
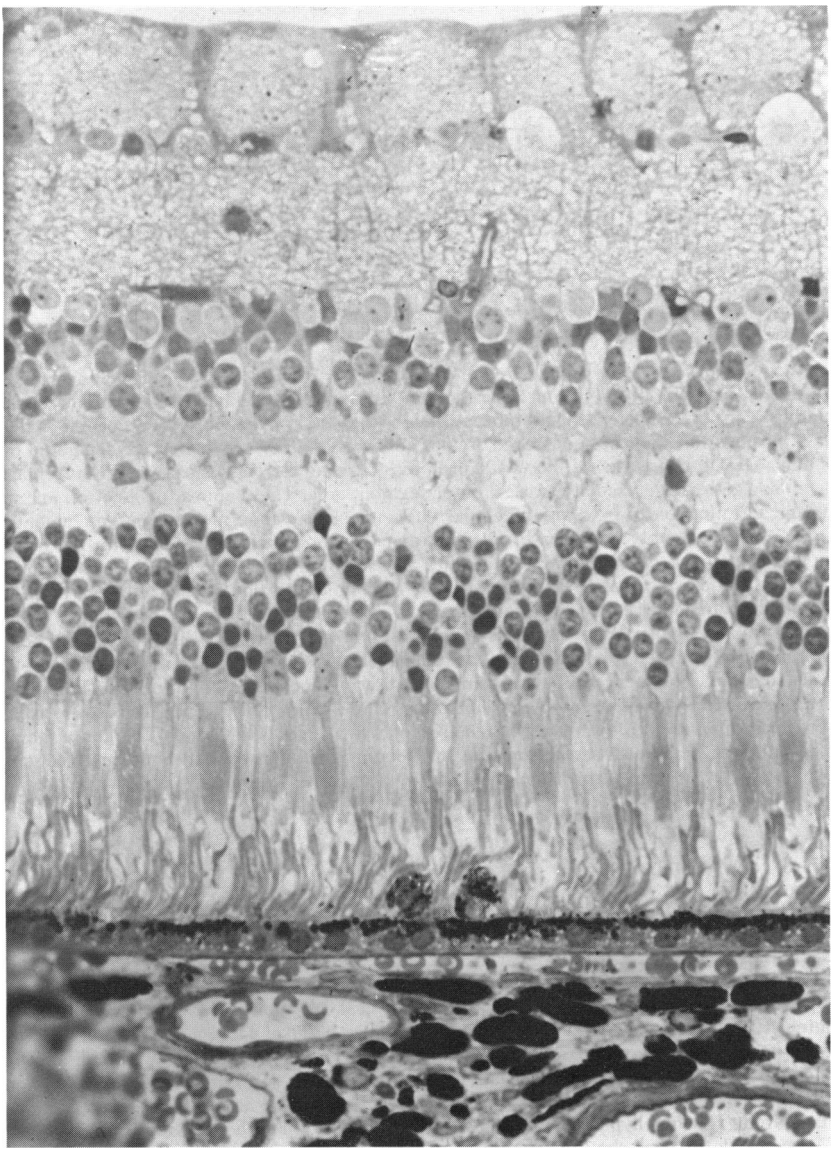


FIGURE 7
Eye exposed to 457.9-nm light for one hour at 0.3 log units above average threshold intensity for this wavelength and duration. Animal was killed at 4.5 months. Overall damage score was 3+. Section shows extreme disformation and destruction of inner and outer segments and loss of pigment epithelium in this area. Note many pigmented macrophages. There is scattered pyknosis in outer nuclear layer that is reduced in width because of cell loss. Pyknosis of cone cells is especially prominent. Many nuclei of inner nuclear layer are pyknotic. Outer plexiform layer is severely swollen and has hyperchromatic processes (toluidine blue, ×300).

missing in between. This figure demonstrates the rapid transition from a damaged area on the left with abnormal outer segments to almost normal outer segments on the right.

Figure 10 shows cell changes in the inner layers one month following a one-hour, 514.5-nm exposure a little below the average threshold. However, this eye overall had "2.5+" damage. The sections were taken one month following exposure. There are prominent hyperchromatic nuclei in the inner nuclear layer. Processes with condensed cytoplasm are seen in the inner plexiform layer as well as in the ganglion cell layer. These changes are present in regions where the pigment epithelial cells remained intact although depigmented. Many outer segments of both rods and cones are preserved.

Overall, the damage is evenly distributed within the retinal layers except for a tendency with the 457.9-nm exposure to injure the inner



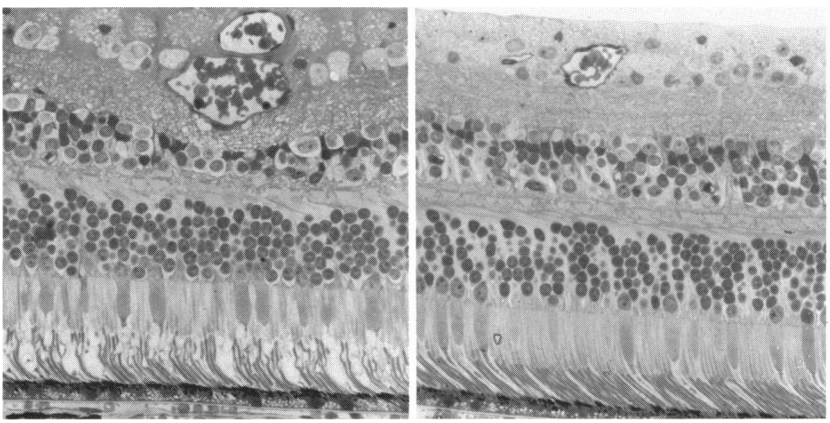
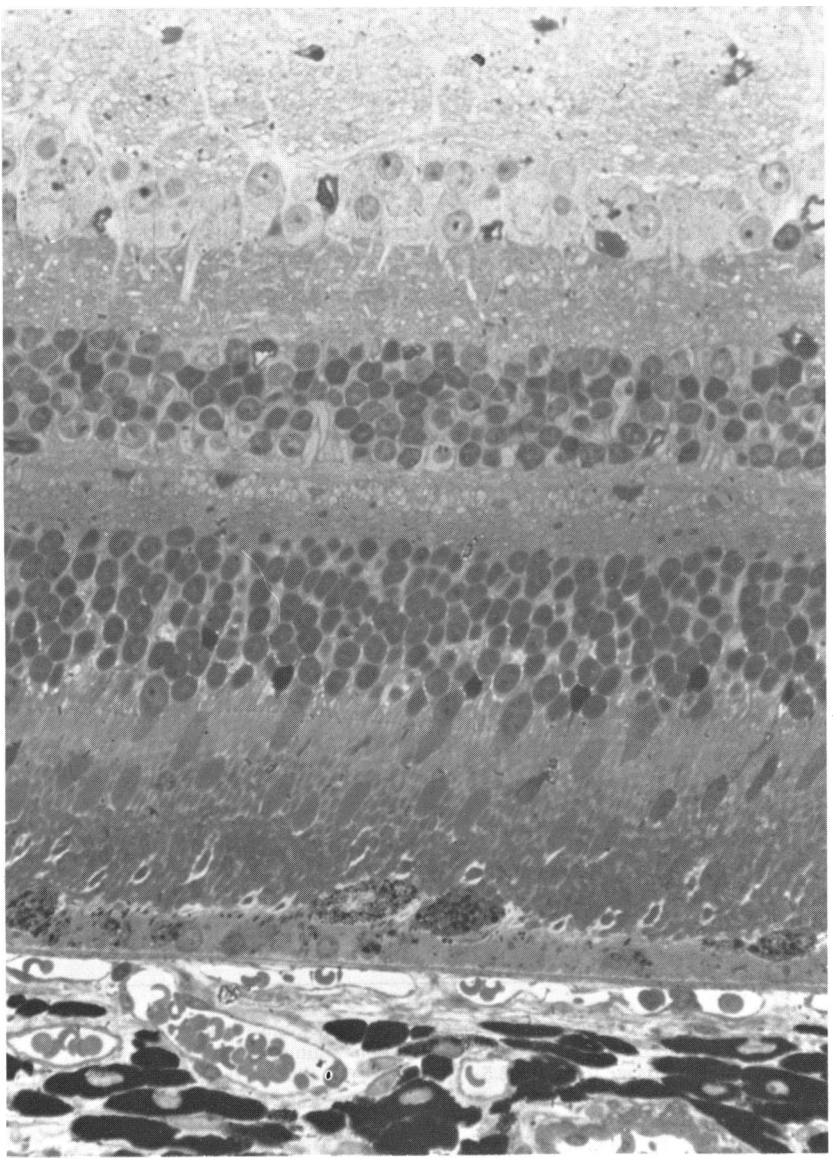


FIGURE 9

Eye was exposed to 476.5-nm light for four hours at approximately average threshold intensity for this wavelength and time. Animal was killed at five months. Two panels in this figure show transition from damaged area to undamaged area. Two panels are separated by one panel width. Left panel shows significant loss of cone cells and those cones that remain are damaged. Large vacuoles exist in outer segment area. These appear on electron microscopic examination to be swollen cellular elements, either cone inner or outer segments. Rods are remarkably intact with only occasional pyknotic nucleus with disturbed outer segments. In this case, essentially all cones appear to be affected. Pigment epithelium in contrast contains normal distribution of pigment in apical portion of cells although there is marked mitochondrial vacuolization. There is significant swelling of elements in outer plexiform and bipolar layer as well as ganglion cell layer. These most likely represent both mitochondria and cell processes. In righthand panel cells show almost no disturbance except vacuolization of pigment epithelium and some disarray in cone outer segments. There may be some mild swelling of outer plexiform layer and Müller cell swelling in bipolar layer. Processes in inner plexiform layer do not appear as swollen as in left panel. This figure shows typical sharp demarcation of damage even though light intensity between these sections does not vary more than 15% (toluidine blue, ×300).

FIGURE 8

Eye was exposed to 457.9-nm light for one hour at 0.3 log units above average threshold intensity for this wavelength and duration. Animal was killed at 4.5 months. Overall damage score was 3+. Microphotograph shows normal choriocapillaris. Pigment epithelium has moderate vacuolization and there are phagocytic cells in subretinal space. Cone inner segments are weakly stained and some appear disformed; some inner rod segments are also disfigured. Heavily hyperchromatic nuclei are present throughout outer nuclear layer. Outer plexiform layer shows dark stained processes as well as swelling. Inner layers show ghosting of cells and swelling of cytoplasm. Receptor cells appear most affected structure in this section. Survival of general retinal architecture is surprising, considering individual cell pathologic changes (toluidine blue, ×300).



layers to a greater degree than with longer wavelengths. An example of this is Fig 11. The eye was exposed for four hours at threshold to 457.9-nm light and removed at three weeks; it showed overall "3.0+" damage. In this section, the pigment epithelium shows little disturbance. Many rod outer segments are still intact. Mitochondria and cell processes are markedly swollen throughout the retina, and hyperchromatic nuclei abound. This picture would suggest that the major insult was not to the outer segments nor the pigment epithelium.

The second question addressed by light microscopy is the time of formation of these lesions as seen several weeks or months after exposure. Sections were taken 1 hour after exposure and 24 hours later in eyes exposed to 1.5 times average threshold of 514.5-nm light for 1 hour.

Figure 12 A was taken one hour after a one-hour exposure. This section shows minimal changes in the pigment epithelium. The outer segments are distorted, and some are swollen. The cone inner segments, however, are markedly enlarged, and sections through the myoid show condensed cytoplasm in some instances. Dark-stained processes of receptor cells course through Henle's fiber layer. Dark cells are seen in the bipolar layer and an occasional dark process in the inner plexiform layer. Figure 12 B shows the foveal area of the same eye. Cell pathologic changes in the outer layers are well demonstrated. These findings are not that much different from those at 24 hours or a month or more following exposure.

Figure 13 is a section from the macula, 24 hours after a one-hour exposure. It shows mild swelling of mitochondria in the pigment epithelium. The cone inner segments are swollen and some nuceli in the outer nuclear layer are pyknotic or hyperchromatic. Condensed cytoplasm is seen in processes in both the outer and inner plexiform layer. These findings are not much different from the findings at one hour or at three months. The small clear vacuoles seen in several layers can be identified as swollen mitochondria by electron microscopic examination. While condensed cytoplasm is noted in the plexiform layers, little swelling is noted here. This might correlate with the fact that retinal translucency

FIGURE 10

Eye was exposed to 514.5-nm light for one hour at 0.2 log units below average threshold intensity for this wavelength and exposure duration. Animal was killed one month after exposure. Overall damage score was 2.5+. Pigment epithelium in this section has lost much of its pigment to phagocytes. Outer segments of both rods and cones appear to be affected. Scattered pyknotic nuclei are in outer nuclear layer. Hyperchromatic processes are seen in outer plexiform layer at border of inner nuclear layer. This eye has been damaged out of proportion to applied dosage and its rod cells have responded to a greater degree than cones, which is not usually seen. One might suspect longer dark adaptation preceding this exposure but this event cannot be determined from records (toluidine blue, ×300).

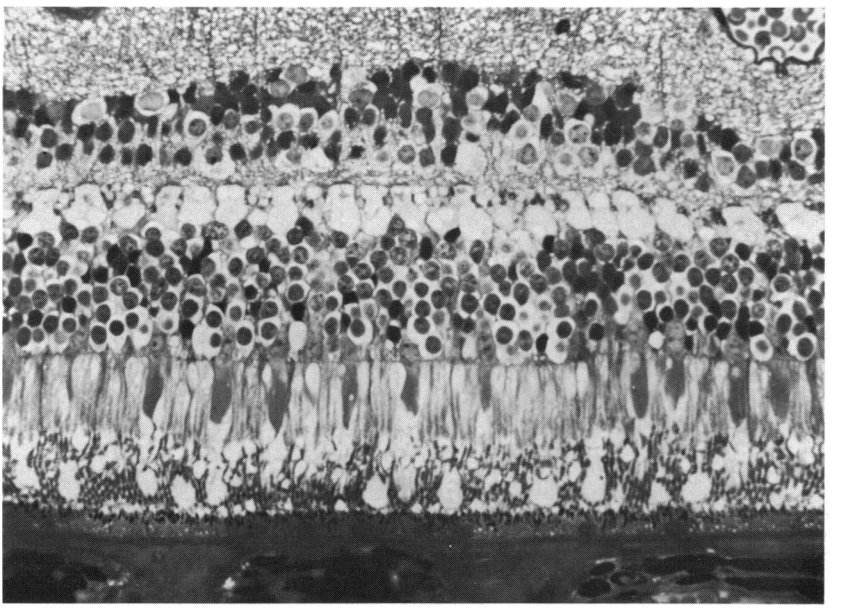


FIGURE 11

Eye was exposed to 457.9-nm light for four hours at average threshold intensity for this wavelength and duration. Animal was killed at three weeks. Overall damage score was 3+. Extreme damage and necrosis is noted throughout section. Cone nuclei and their inner segments are severely disfigured or vacuolized. A great portion of rod nuclei is also pyknotic. There are patches of rod outer segments that appear retained. Pigment epithelium appears devoid of changes. There is extensive swelling of plexiform layers. Inner nuclear layer shows scattered pyknosis and nuclear destruction (toluidine blue, ×300).

only becomes prominent at 72 hours, seen clinically with indirect ophthalmoscopic examination as edema.

Electron Microscopic Evaluation.—Electron microscopic section of light-damaged eyes usually shows in every retinal layer mitochondrial swelling of some degree. In many mitochondria the cristae are destroyed. Figure 14 shows typical mitochondrial destruction in the pigment epithelial cells. These pigment epithelial cells also show a partial shift of pigment away from the apical border. The rod outer segments are distorted, but many are intact at this time three weeks after exposure.

The section in Fig 15 shows the vacuolized mitochondria as part of the cone inner segments. Many more of the long, thin-rod mitochondria retain a few of their cristae and are generally better preserved than the thicker-cone mitochondria. Mitochondria contained in receptor cell processes in the outer plexiform layer appear to be swollen or disorganized

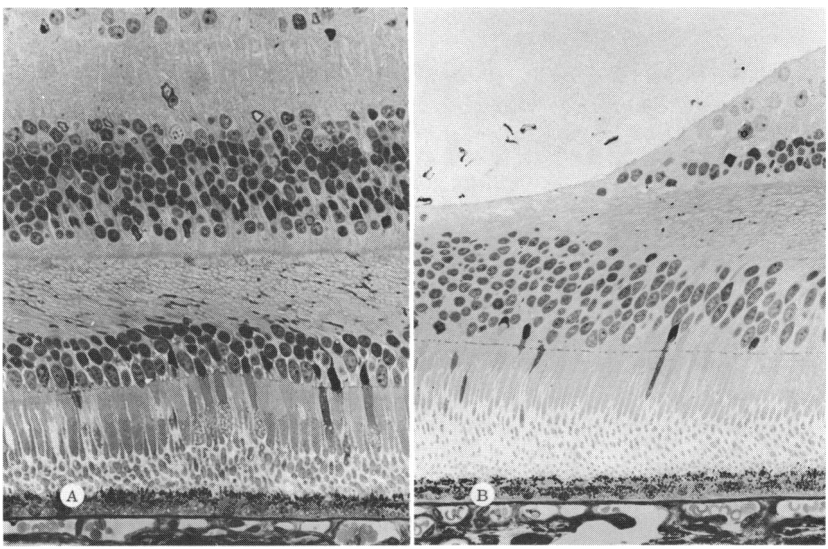
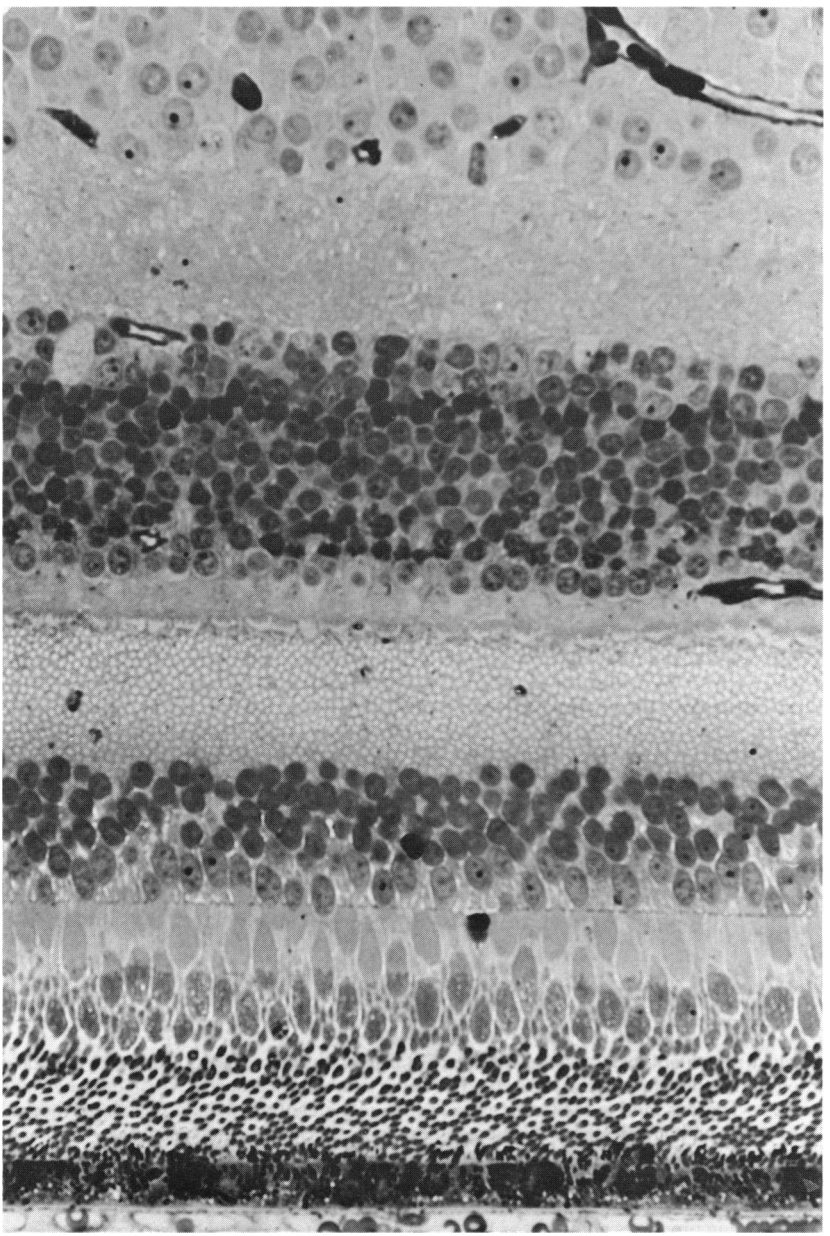


FIGURE 12

A: Eye was exposed to 514.5-nm light for one hour, approximately at threshold, and immediately removed. This section was taken from macular region. There is some disturbance of pigment and mild vacuolization in pigment epithelium. Outer segments appear swollen and somewhat shortened. Inner segments in this cone-rich area are swollen, often darkly stained, and vacuolized in their distal half. Many nuclei of outer nuclear layer are pyknotic. Condensed (dark) cytoplasm extends into Henle's fiber layer up to synaptic region. There is abnormal cellular staining in inner nuclear layer, and inner plexiform layer shows a few darkened processes. Ganglion cells are fairly well preserved. These changes seen 1 hour after exposure are not much different from those seen at 24 hours or several months (toluidine blue, ×650). B: Section through fovea of same eye. Overall damage grade was 2.5+. Cellular changes are prominent in ganglion cell layer, inner nuclear layer, and very prominent in outer plexiform layer. There are scattered, dark-cone nuclei; a few cone inner segments also are hyperchromatic. No significant changes are seen in pigment epithelium (toluidine blue, ×650).

(Figs 16 and 17); synaptic vesicles can still be seen and the cells may continue to function. Mitochondrial swelling may or may not be associated with condensed chromatin of the cytoplasm (Fig 17). Cellular disorganization is also evident in the nuclear region of the receptor shown by pyknosis, fading, and cellular organelle swelling (Fig 18).

Similar mitochondrial changes as in the receptor cells are found through the bipolar and inner plexiform layers. This is shown in Figs 19 and 20. The synthetic machinery of these cells appears intact and possibly hyperactive if one can judge by the appearance of the endoplasmic reticulum and Golgi body.



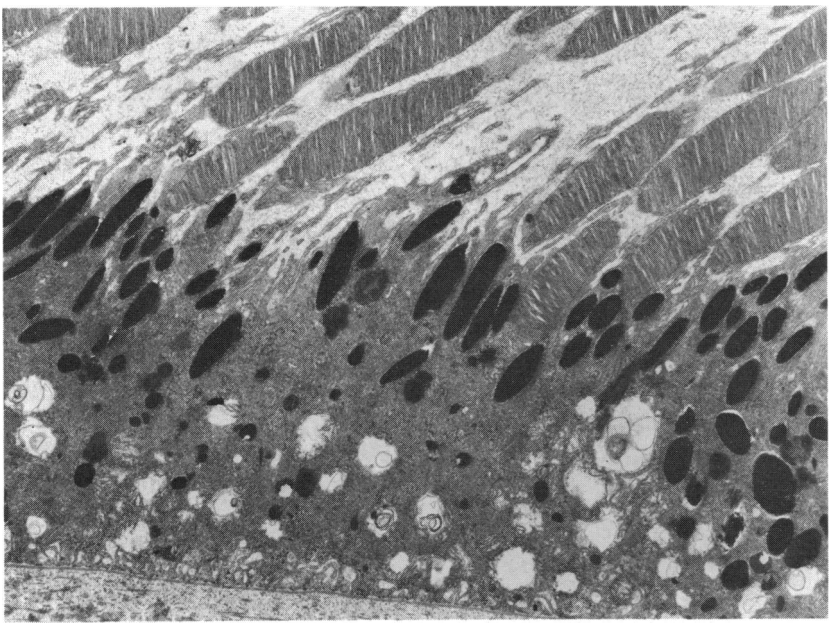
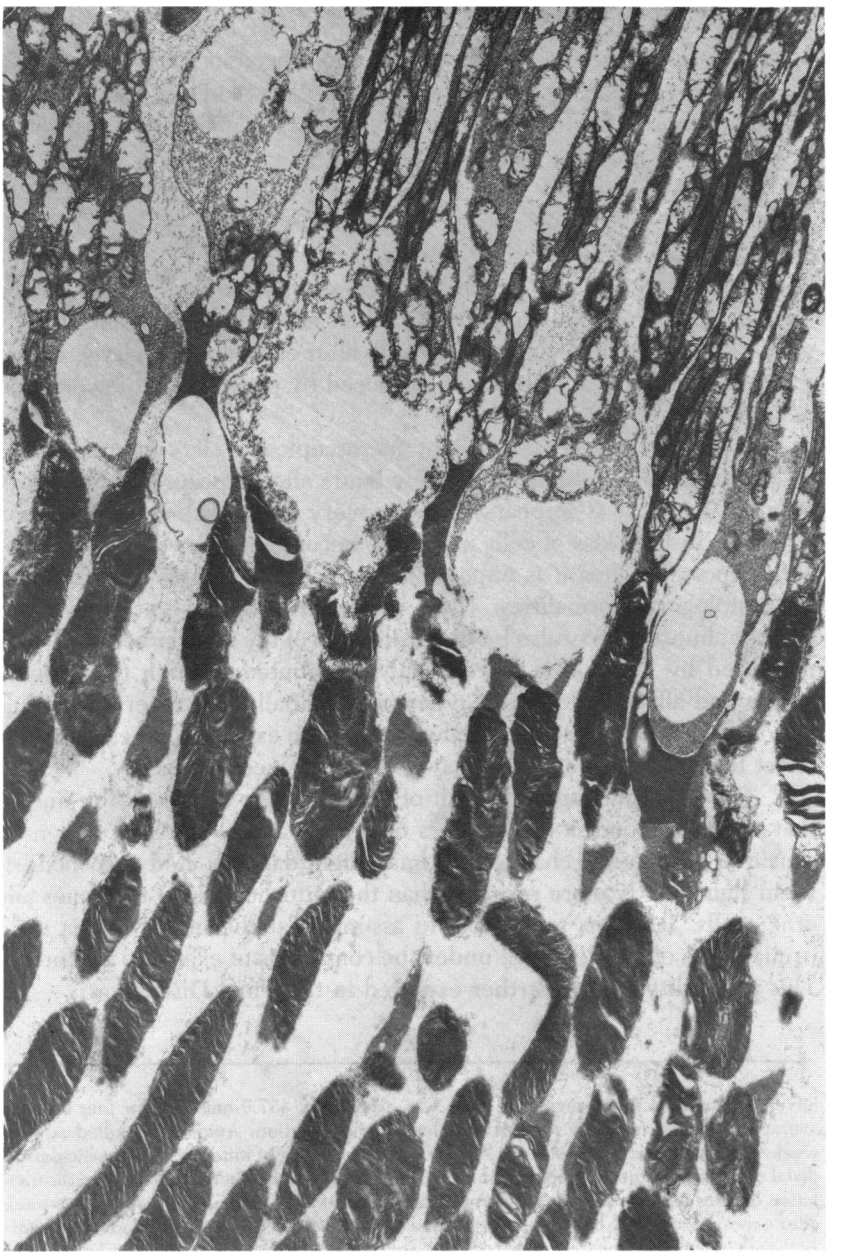


FIGURE 14

Pigment epithelium, mild form of damage. Eye was exposed to 514.5-nm light four times at 1 hour each, at 24-hour intervals, at 0.2 log units less than average threshold intensity for this wavelength. Animal was killed at one month following exposure. Overall damage score was 2+. This photomicrograph shows very mild changes noted in pigment epithelium from light damage. Mitochondria are swollen and cristae obliterated. There is mild increase in number of residual bodies and there are halos around some lysosome bodies. Basal enfolding is intact and only very slightly swollen. Normal cigar-shaped pigment granules are aligned along apical surface of cell, although villi are somewhat shortened. There are few instances where pigment granules are located more deeply in cell (×7200).

FIGURE 13

Macular region of this eye was exposed to 514.5-nm light for one hour at average threshold intensity for this time. Animal was killed 24 hours following exposure. Overall damage grade was 2+. There is mild to moderate vacuolization of pigment epithelium, but this is difficult to evaluate without electron microscopic examination. Outer segments of rods and cones appear grossly intact. Inner segments of cones are thicker than normal and a few are abnormally dark. There are scattered hyperchromic nuclei in outer nuclear layer and some swelling. Processes of outer plexiform layer are uniform in size. However, there are several dark-staining processes noted out as far as synaptic region. Scattered hyperchromic cells are seen in inner nuclear layer and ganglion cell layer. These findings are not that different from those seen at shorter or longer survival times (toluidine blue, ×650).



Swollen mitochondria and swollen processes are also seen in cells of the ganglion cell layer (Fig 21) and in the nerve fiber layer (Fig 22).

The electron microscopic finding of outer segment swelling, vesiculation of discs, swelling of cellular organelles other than mitochondria, and many other changes throughout the retinal layers have been previously described by Lawwill et al[11] and others.[24,40]

DISCUSSION

The following findings deserve emphasis:

1. The changes observed with electrophysiologic testing and clinical observation are to some degree reversible. Electroretinographic changes generally become less within two weeks after exposure. Similarly, edema disappears while fundus changes produced by pigment epithelial abnormalities tend to persist.

2. Histologic changes at the light microscopic and electron microscopic levels, however, are as marked a few hours after exposure as after three months. At least it appears that recovery from histologic change is minimal. In fact, loss of cells may have become more evident with time after exposure. Thus it is impossible to relate the functional changes to the histologic abnormalities. The two may involve different pathways of damage, but they may also be the expression of the same process; the one measured by function is more evenly distributed through the exposed region and affects certain cell types preferentially and reversibly, while the others seen by histologic evaluation are the extreme expression of the effect that is scattered over all layers and many cell types.

3. The most outstanding result of the histologic studies is the finding that the mitochondria of all layers of the retina are the structure most sensitive to anatomic change in primate light damage. Rod mitochondria seem significantly more resistant than the mitochondria of the cones and other cells. It seems reasonable to assume that the mitochondria are a primary site of light damage under the conditions of exposure as studied. This possibility will be further explored in the Final Discussion.

FIGURE 15

Severely damaged inner segments. Eye was exposed to 457.9-nm light for four hours at average threshold intensity for this wavelength and duration. Animal was killed at three weeks. Overall damage score was 2.75+. Extreme swelling of mitochondria, particularly of distal end of cone inner segments, is noted in this photograph. There is also severe disorientation of discs in outer segments. These discs may have been assembled during three weeks after exposure and may have been misformed because of severely damaged inner segments. Rod cells seem less severely affected, but some show condensation of cytoplasm. Dark-staining inner segments are attached to outer segments in at least one instance (×5200).

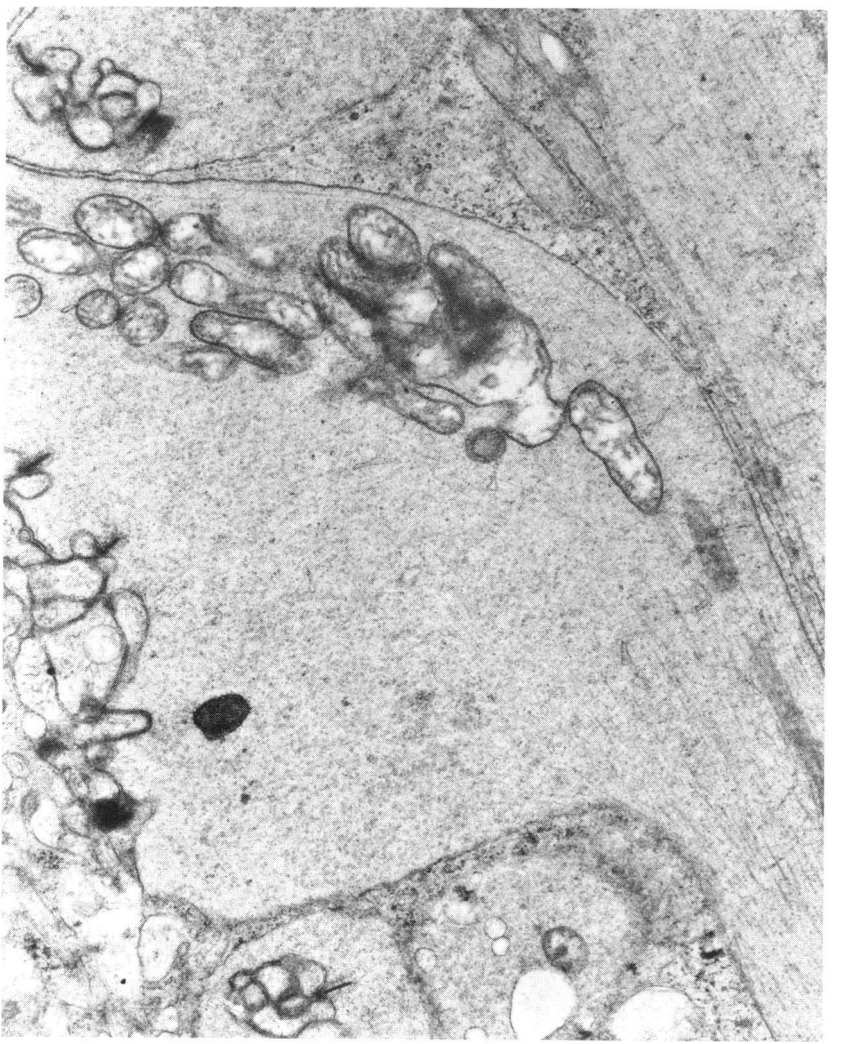


FIGURE 16

Outer plexiform layer, damaged cone pedicle. Eye was exposed to 476.5-nm light for four hours at approximately average threshold intensity for this wavelength and duration. Animal was killed at five months. Overall damage score was 2.5+. This photograph shows cone pedicle with mitochondria showing swelling and lack of cristae. No severe damage is noted in this area of an otherwise severely damaged retina (× 15,600).

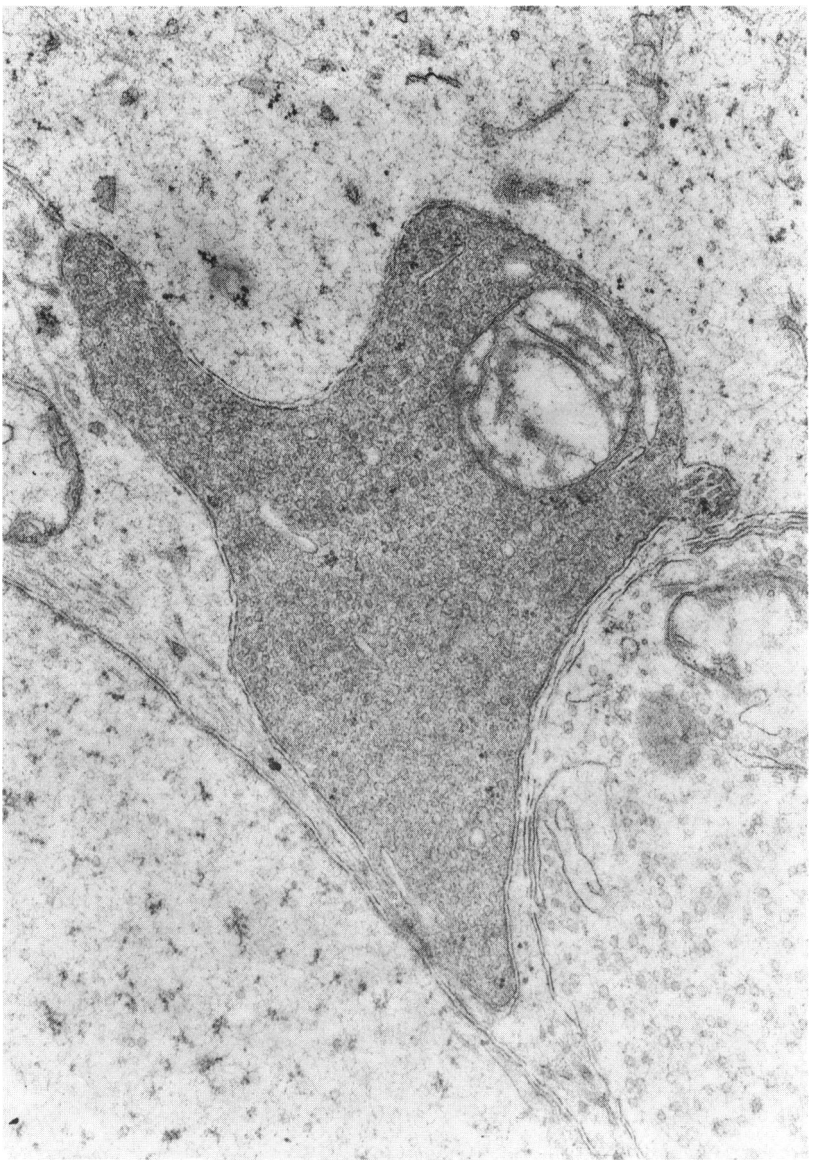


FIGURE 17

Outer plexiform layer, condensed cytoplasm. Eye was exposed to 457.9-nm light for one hour at 0.3 log units above average threshold intensity for this wavelength and duration. Animal was killed at 4.5 months. Overall damage score was 3+. This is photograph of cell process in outer plexiform layer that shows extensive condensation of cytoplasm. Moderately swollen mitochondria are present in this process. Cell membrane appears intact (×42,380).

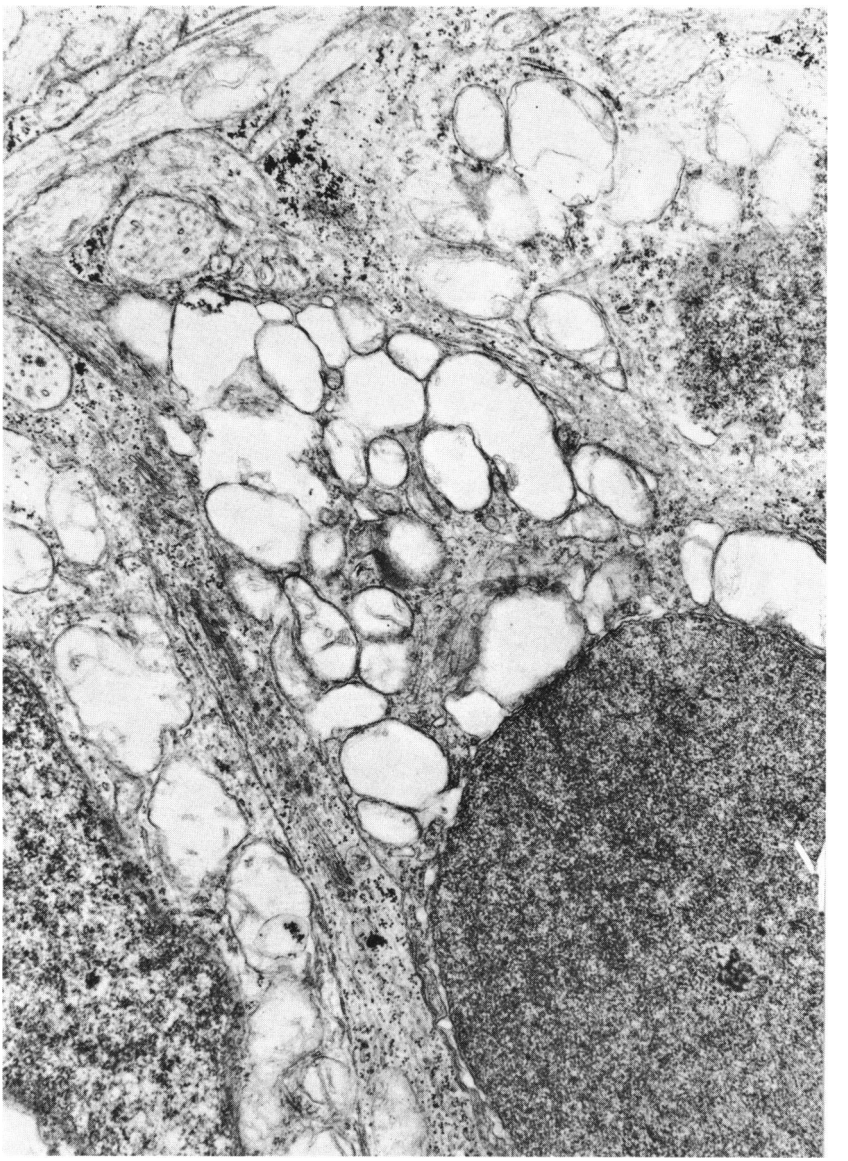


FIGURE 18

Mitochondrial damage in bipolar cell layer. Eye was exposed to 514.5-nm light four times at 1 hour each, at 24-hour intervals, at 0.2 log units less than average threshold intensity for this wavelength. Animal was killed one month following exposure. Overall damage score was 2+. This section demonstrates extensive destruction of mitochondria in neural processes. They are swollen and missing their cristae. A Müller cell process with unusual accumulation of glycogen coursing through section (TEM, ×18,000).

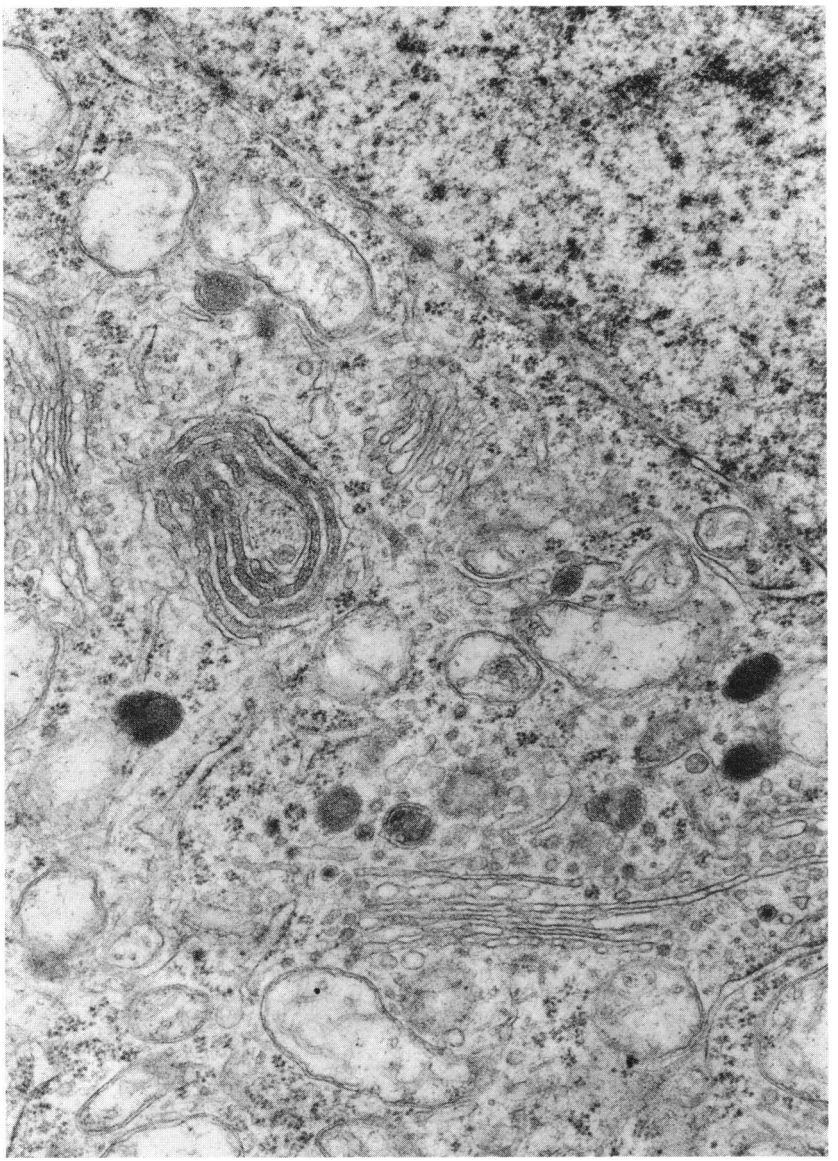


FIGURE 19

Enhancement of smooth and rough endoplasmic reticulum (ER) in bipolar cell layers. Eye was exposed to 514.5-nm light for one hour at 0.2 log units below average threshold intensity for this wavelength at this time. Animal was killed at one month. Overall damage score was 2.5+. This section through bipolar layer shows increased activity of both smooth and rough ER. Most mitochondria are severely swollen with loss of cristae. Lysosomal bodies are more frequent than is normal (TEM, ×35,620).

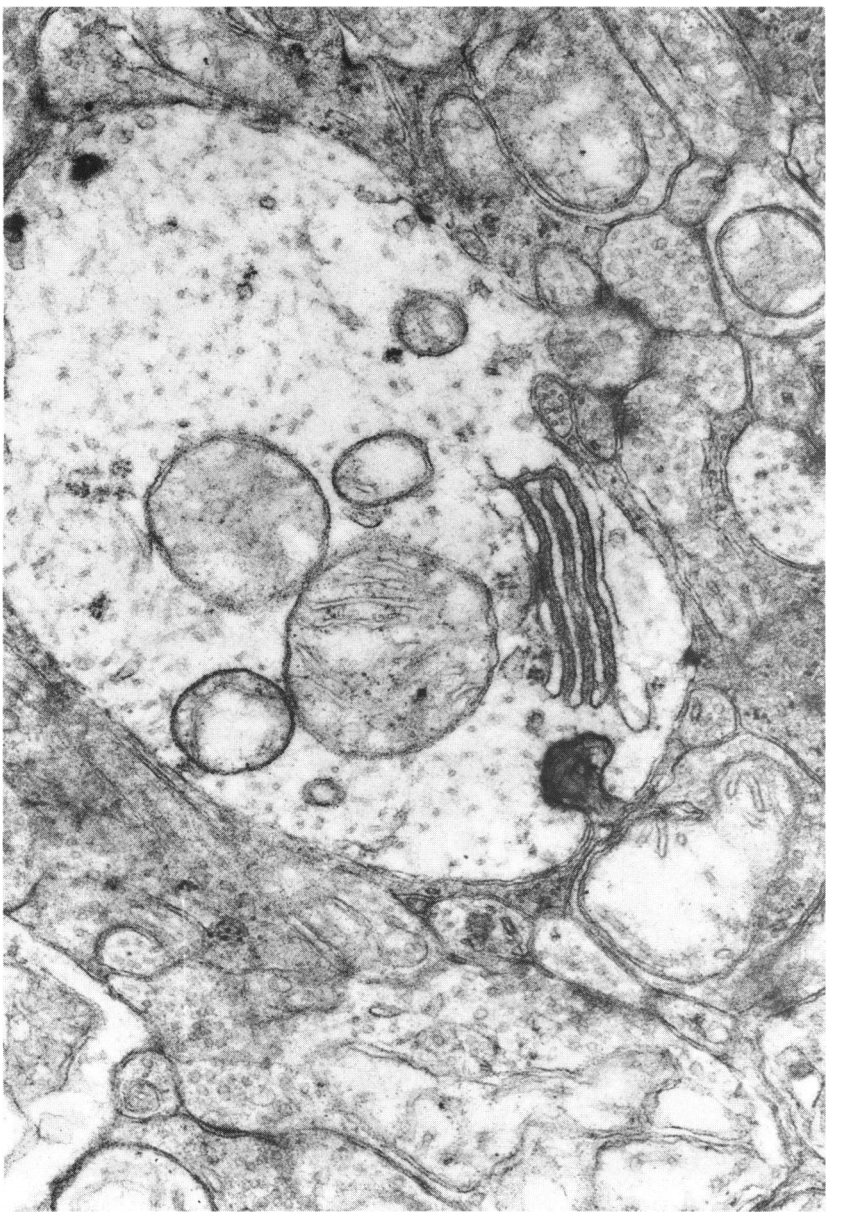


FIGURE 20

Inner plexiform layer. Eye was exposed to 476.5-nm light for four hours approximately at average threshold intensity for this wavelength and duration. Animal was killed at five months. Overall damage score was 2.5+. This photograph shows normal and swollen mitochondria and significantly swollen cell with evidence of metabolic hyperactivity signified by pattern of rough endoplasmic reticulum (×42,380).

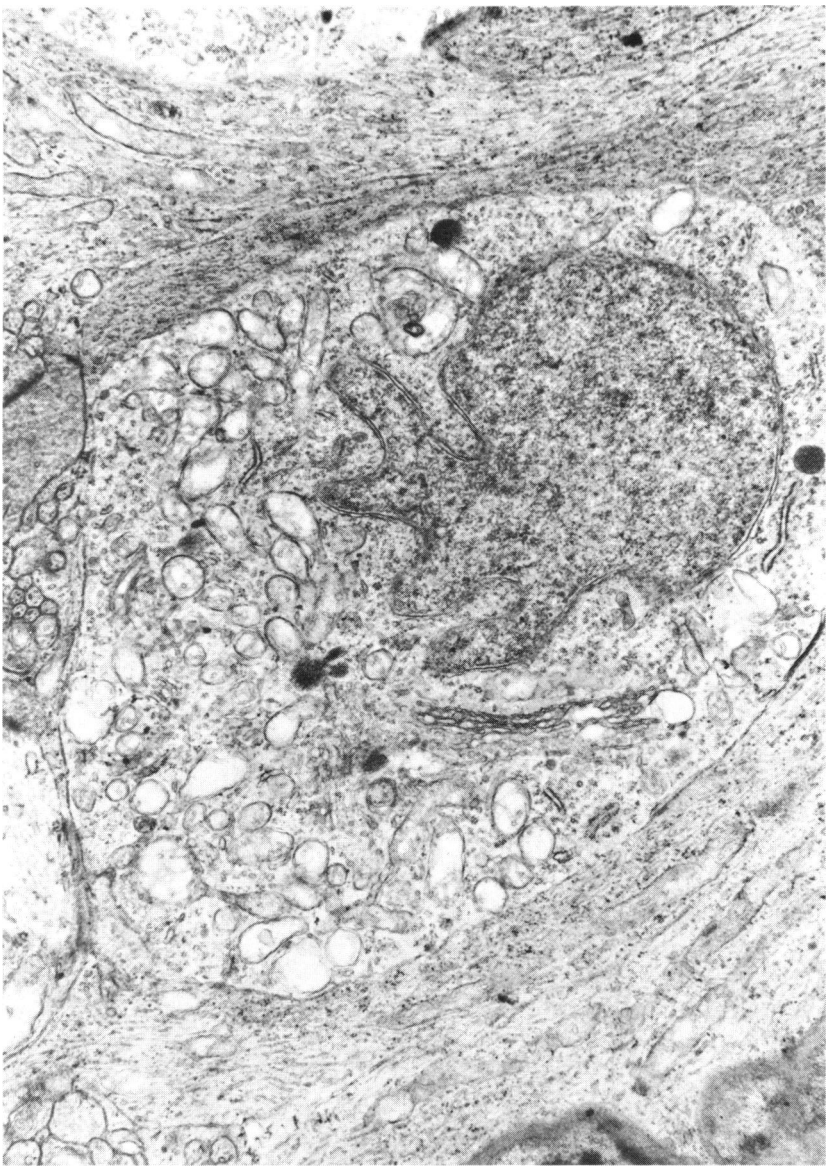
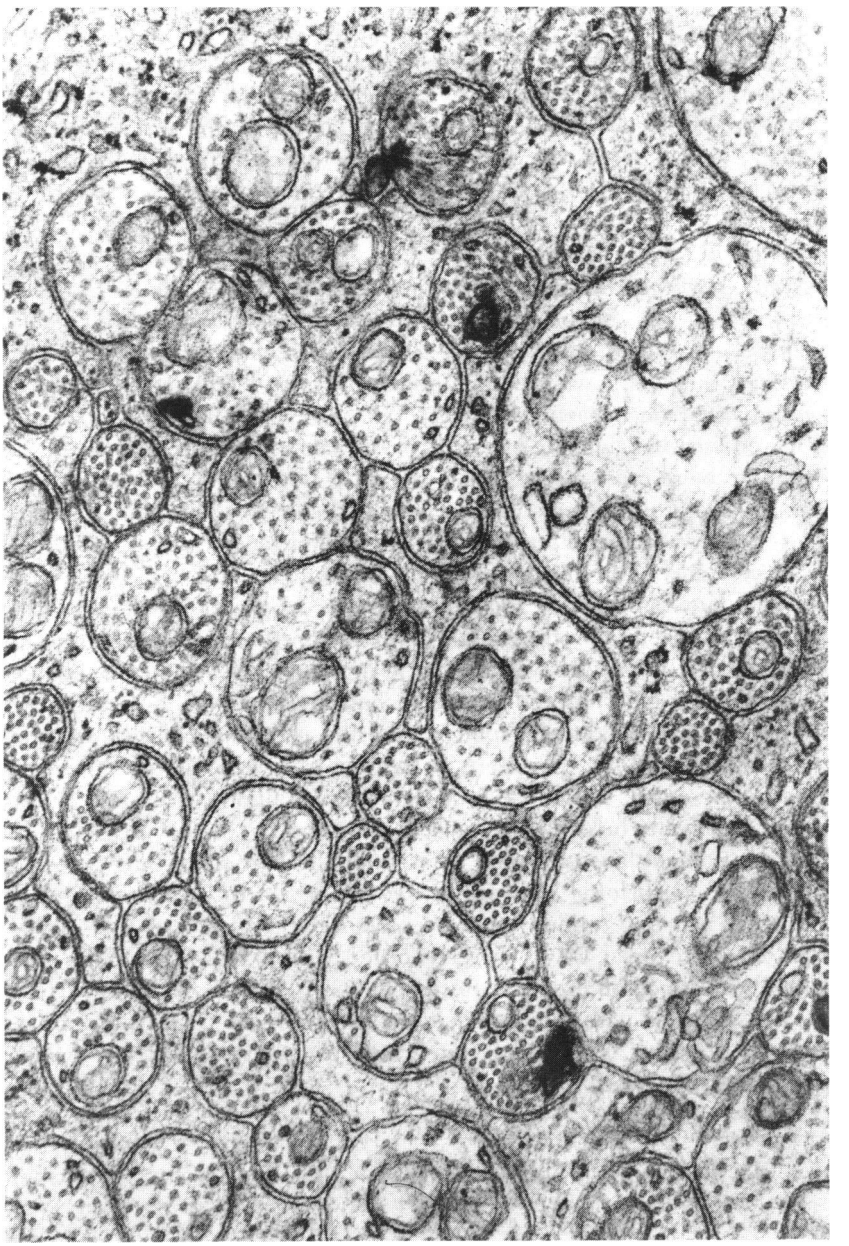


FIGURE 21

Ganglion cell layer. Eye was exposed to 476.5-nm light for four hours approximately at average threshold intensity for this wavelength and time. Animal was killed at five months. Overall damage score was 2.5+. Cell shown was found in ganglion cell layer. There is swelling of mitochondria and other intracellular organelles. Cytoplasm appears pale and somewhat empty. This is one of milder types of damage seen in ganglion cell layer (×15,600).



EXPERIMENT 2: SPECIFIC CONE DAMAGE IN THE MONKEY RETINA

Experiment 2 describes an elaborate attempt to determine whether cone cells are specifically susceptible to wavelengths of light that are absorbed by their specific visual pigment. Following exposure to 451.9 (blue), 514.5 (green), or 590 (red) nm of laser light covering 40° of the posterior retina, spectral sensitivity functions were obtained by electroretinography using as the stimulus an alternating monochromatic bar pattern.

METHODS

Animals, Exposures.—Six mature female cynomolgus monkeys (*Macaca fascicularis*) were obtained by the Carolyn Verhoeff animal care center. They were housed in cages with a 12-hour day–12-hour night cycle. Food (Purina Monkey Chow, supplemented with fresh fruits and vegetables) was provided once each day in the late afternoon. The animals were prepared for the damaging exposure in the same way as in experiment 1. Four eyes were randomly assigned to each of three argon laser treatment groups. One group received a four-hour exposure to 457.9-nm laser light at a retinal irradiance of 5 mW/cm^2. The second received 514.5-nm light at 10 mW/cm^2. The third group was exposed to 590-nm light at 40 mW/cm^2. Irradiances for exposure were based upon the best estimates for those required to produce a just-greater-than-threshold damage. As in experiment 1, laser light was presented in Maxwellian view, providing a retinal field of 40° centered on the macula. Retinal position was monitored with a handheld beam splitter. Anesthesia during exposure was the same as described in experiment 1. Heating pads were used to maintain body temperature, which had a tendency to fall under barbiturate anesthesia. The eye to be exposed was held open and the cornea protected by the same contact lens electrode as used for the ERG recording.

Spectral Sensitivity Measurements.—Electroretinographic spectral sensitivity was determined in 12 eyes before and after laser light exposure. Phase reversal ERGs were recorded with a lock-in amplifier and voltmeter (Princeton Applied Research, 5204). The measuring system was phase-locked with the 8-Hz pattern reversal. The response in phase with the stimulus change was integrated over a three- or ten-second period and displayed.

FIGURE 22

Nerve fiber layer. Eye was exposed to 476.5-nm light for four hours approximately at average threshold intensity for this wavelength and duration. Animal was killed at five months. Overall damage score was 2.5+. This cross section of nerve fibers shows relatively normal fibers mixed with others that have swollen mitochondria and pale cytoplasm. This photograph represents lesser grade in variable range of observed reactions (×50,444).

The bar-pattern target was a celluloid film with alternating light and dark horizontal bars with a contrast between the light and dark bars of 1.6 log units. Alternate vertical movements of the target reversed the position of the dark and light bars, providing local changes in retinal illumination resulting in the ERG signals. The method was essentially that of Johnson et al.[41] Eight wavelengths with 10-nm band width were provided by a grating monochromator (Bausch and Lomb 33-80-02). The wavelengths selected were 460, 490, 520, 560, 595, 610, 640, and 685 nm. The target field of 38° diameter was presented to the eye in Maxwellian view so that it was centered on the macula. Each pattern cycle of the target subtended 2.72°, ie, the spatial frequency was 0.367 cycle per degree. Position of the pattern on the retina was monitored with a beam splitter.

A neutral density filter was used to adjust the amount of light entering the eye until a 0.75 μV criterion response was measured on the lock-in voltmeter. The retinal irradiance necessary to produce the ERG criterion response was measured three times during each experimental session. Four separate sessions at four-day intervals were used to evaluate the ERG spectral sensitivity both before and after laser light exposure.

The animals were prepared for recording by phencyclidine hydrochloride injected IM (1 mg/kg^{1}) to produce a "dissociated" state. The eyes were dilated and the animal was placed in a chair designed to position the subject into Maxwellian view. After endotracheal intubation with local anesthesia, the animal was immobilized with intravenous (IV) gallamine triethiodide (5.3 mg/kg^{1}) and positive-pressure ventilation was adjusted to achieve 4% end tidal CO_2. Immobilization was maintained with an IV infusion of gallamine in saline (2.8 mg/ml^{1}) at a rate of 0.05 ml/min^{1}. At the conclusion of the two-hour experimental session, the animal's recovery was assisted by injection of atropine sulfate (0.13 mg/kg^{1}) and neostigmine methylsulfate (0.3 mg/kg^{1}). It was not unusual to observe the monkey eating biscuits and fruit 30 minutes after the end of the recording session.

The use of a neuromuscular blocking agent was based upon the need to restrain the animal, insert contact lens electrodes in each eye, and eliminate eye movements. Initially phencyclidine alone was used. However, this induced a vertical nystagmus that made recording of pattern evoked responses and ERG impossible. The inhalation anesthetic, methoxyfluorane, was then tried. This agent was not suitable for a two-hour procedure because of its nephrotoxicity. A review of the literature revealed that pentobarbital altered components of the ERG depending upon the level of anesthesia. Nitrous oxide was not used because of the difficulty of maintaining adequate oxygenation without the availability of blood gas

measuring equipment. The procedure we finally chose allowed reliable ERG measurements while meeting the ethical standards in the humane treatment of the monkeys.

RESULTS

The results are presented in Fig 23 as the log relative spectral sensitivity for all 12 eyes before exposure and for each of the three treatment groups after exposure. After exposure, one of the eyes exposed to the 457.9-nm light and one of the eyes exposed to the 590-nm light could not generate the criterion level ERG, so only three eyes represent each of these groups.

As Fig 23 shows, the relative sensitivity to the 460-nm test light was most reduced after the 457.9-nm exposures. On the other hand, the great reduction in sensitivity after 514.5-nm exposure was revealed for the 520-nm and 560-nm pattern lights. Relative sensitivity to 610- and 640-nm test lights was most reduced after the 590-nm exposures. It is also significant that the least reduction in sensitivity to the blue portion of the spectrum was found after the 590-nm exposures. Similarly, the least reduction in sensitivity to the red test lights was found after the 457.9-nm exposures.

Figure 24 demonstrates the changes in spectral sensitivity as a difference between the preexposure and postexposure curves. This presentation clearly shows that the spectral region with the greatest decline in sensitivity corresponds to the color quality of the damaging light, ie, blue, green or red. This is in addition to the fact that the blue 457.9-nm exposure required ⅛ or less the intensity of the 590-nm exposure to achieve a similar level of damage.

DISCUSSION

The findings of experiment 2 clearly demonstrate a damage mechanism dependent individually upon the cone pigments in addition to or on top of a separate damaging effect of light which has an action spectrum peaking at the short-wavelength end of the spectrum. The spectral effect most closely relates to Sperling's damage of specific cones that occurred at two to three log units lower intensity. However, at those intensities he was unable to "red blind" his animals; in our experiments relative red blinding did occur, but only in the presence of nonspecific destruction. Experiment 2, then, is an example of a mixture of mechanisms or pathways to damage.

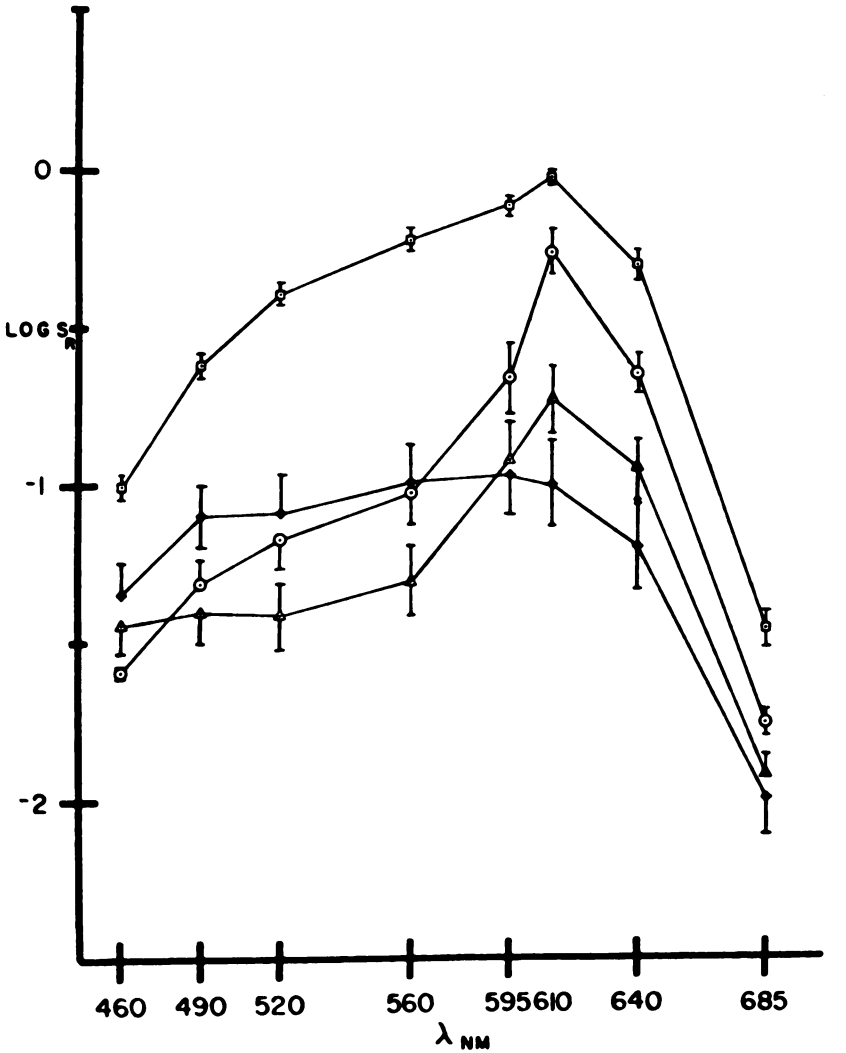


FIGURE 23

Spectral sensitivity function. Log sensitivity (see text) is plotted against wavelength of stimulating light. Spectral sensitivity (12 eyes) was determined before exposure (squares) and after exposure to each of three wavelengths (590 nm: filled diamonds; 514.5 nm: triangles; 457.9 nm: circles). Exposure at 457 nm reduced electroretinogram sensitivity to 460-nm test light to greater extent than did exposure at other wavelengths. Sensitivity to test lights from 490 to 560 nm was most affected by exposure at 514.5 nm, while sensitivity to test lights from 595 to 685 nm was most effectively reduced by exposure at 590 nm.

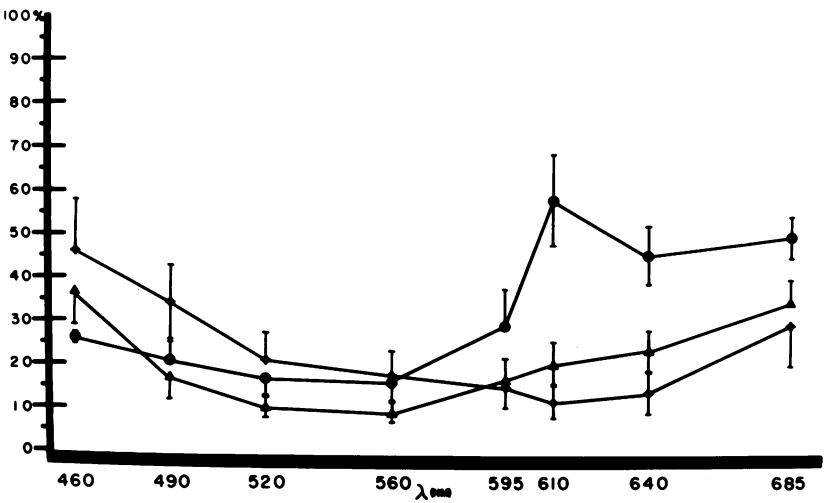


FIGURE 24

Difference spectrum of electroretinogram sensitivity between preexposure and postexposure measurements. Percent loss in spectral sensitivity is calculated for each exposure group. This analysis shows that (1) 590-nm exposure (diamonds) selectively reduces sensitivity in long-wavelength region of visible spectrum; (2) 514.5-nm (triangles) exposure preferentially reduces sensitivity in midrange wavelengths; and (3) 457.9-nm (circles) exposure decreases sensitivity throughout short and midrange region with considerably less effect in red.

EXPERIMENT 3: LIGHT DAMAGE IN CULTURE OF BOVINE RETINAL PIGMENT EPITHELIUM

This study is an attempt to determine whether the light damage of the retinal pigment epithelium (RPE) is primary or secondary. Secondary damage refers to the possibility that the pigment epithelial damage occurs in response to changes in neighboring tissue, the photoreceptor cells, or the choroid. The question asked was whether light at a similar intensity as in vivo produces a pigment epithelial effect in vitro, and which wavelengths are most effective.

METHODS

Collection of Cells.—Fresh cow eyes were obtained from the local slaughterhouse and transferred on ice to the laboratory. The ophthalmic artery was cannulated, and the eyes were infused with a solution consisting of protease, collagenase, and trypsin. Ten minutes later the anterior segment was removed, and the retina was dissected free of the eye cup. The RPE cell layer was removed from the eye cup by a gentle flushing and aspiration action with 0.1% EDTA solution. The final effluent was aspirat-

ed, and the solution was centrifuged. Cells were washed several times with cold media, then resuspended and distributed to 20-ml petri dishes. The primary cultures were incubated in a CO_2 water-jacketed incubator, and within seven days they had grown to confluency. At this time the cells were resuspended, collected, distributed to 15-ml petri dishes, and returned to the incubator. Within approximately five days these first-order subcultures had grown to confluency (Fig 25). The cells were maintained on Eagle's minimal essential media in a bicarbonate buffer. The medium was changed twice each week.

The cow eye is composed of tapetal and nontapetal regions. The RPE in the tapetal area lacks melanin granules (Fig 26), while the nontapetal region has a pigmented RPE (Fig 27). By dissecting these areas apart, RPE cells with and without melanin were successfully obtained. This differential harvesting technique has been utilized to produce melanin-containing and melanin-free cell cultures.

Exposure of Cell Culture.—In one procedure, confluent monolayer subcultures of mixed tapetal and nontapetal cells were exposed to the

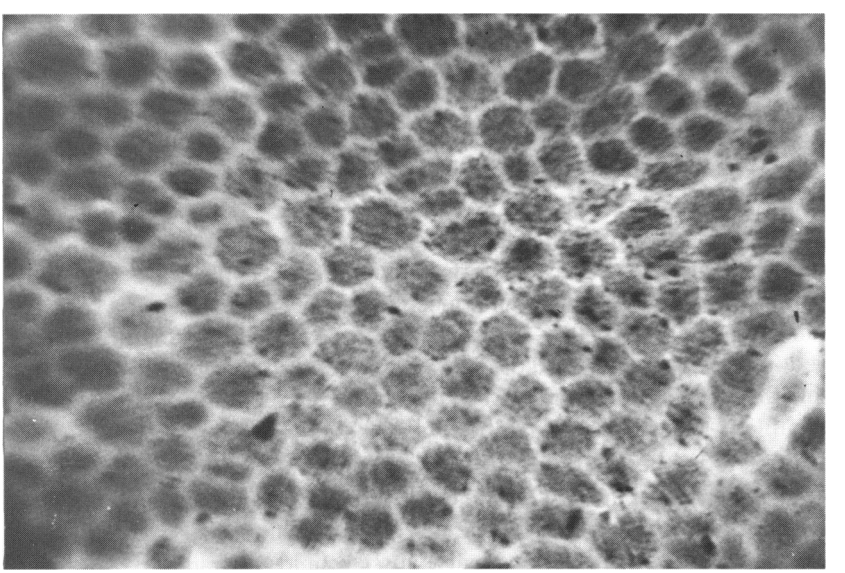


FIGURE 25

Bovine retinal epithelial cells in culture. Ten days after first passage cells have polygonal morphologic features characteristic of in vivo RPE. Culture shown is from cell harvest including tapetal and nontapetal areas. The degree of pigmentation is more sparse than that observed in tapetal cells in vivo. Brightfield illumination, fixed and stained with cresyl violet (×700).

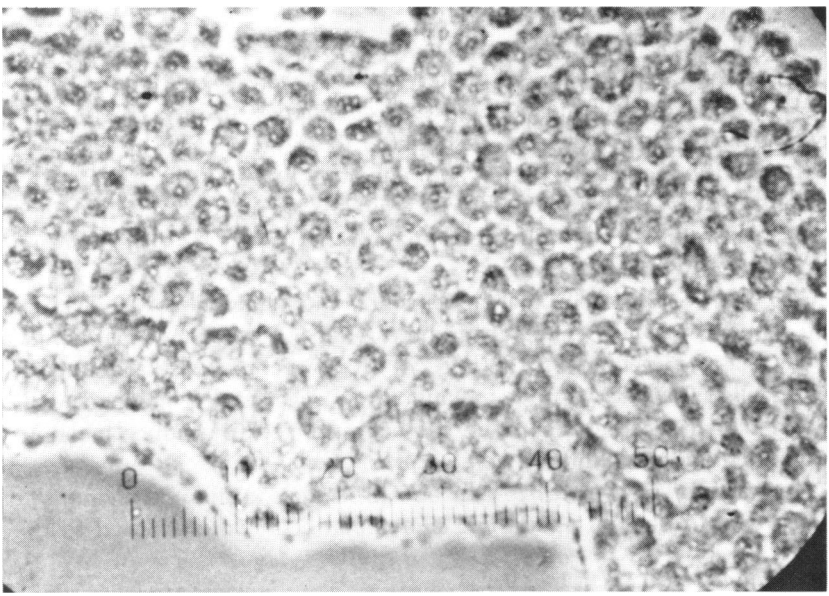


FIGURE 26
Bovine retinal pigment epithelium from tapetal region of retina. Tissue was dissected free of Bruch's membrane under operating microscope after removal of neural retina. Few melanin granules that are present appear as bright flecks under phase microscope. The tissue, an explant, was observed ten minutes after its removal from eye (unfixed, phase contrast, ×525).

457.9-nm or 514.5-nm lines of the argon laser, or the 590-nm emission of the rhodamine 6-G dye laser, for two hours. During exposure the cultures were maintained at 37° C in an atmosphere of 5% CO_2 in air at a relative humidity of 95%. Twenty-four hours after exposure the cultures were fixed in phosphate-buffered 3% glutaraldehyde and stained with 1% toluidine blue-O. No precautions were taken to avoid exposure of the cultures to ambient laboratory illumination when being transported.

A knife-edge beam-stop was placed along a cross-sectional diameter of the laser beam and imaged on the culture plate. This technique produced a hemifield exposure pattern. The lasers were maintained in the TEM_{oo} mode, and thus a two-dimensional Gaussian field of irradiance was provided. The spatial distribution of irradiance was mapped using a light detector with a 1-mm aperture.

In a second procedure, groups of pigmented or nonpigmented cells were exposed for two hours to the broadband filtered (335- to 600-nm) light from a mercury-xenon lamp.

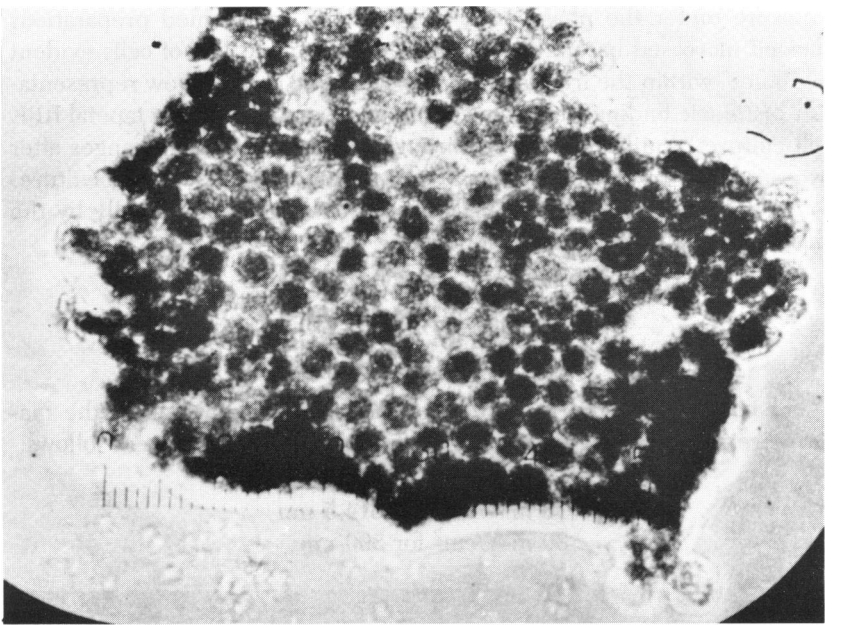


FIGURE 27
Bovine retinal pigment epithelium explant from nontapetal region of retina. As in Fig 26, explant was dissected free of Bruch's membrane under operating microscope after removal of neural retina. Cells contained dense population of melanin granules, which in sufficient quantity appear as opaque areas over cell. Those cells with fewer granules are observed as varying shades of gray. Samples taken from more peripheral regions contain more melanin (unfixed, phase contrast, ×525).

The effects of light exposure were determined by histologic evaluation of stained preparations with light microscopy. Correlations between the locus of observed histopathologic changes and exposure irradiance were made possible by the precise registration of the culture plate, both in the exposure chamber and on the microscope stage. The geometry of the irradiance field allowed each culture to serve as its own control, ie, unexposed regions were adjacent to areas receiving a graded irradiance. Thus an entire dose-response function could be obtained from a single culture.

RESULTS

Exposure of RPE cell cultures to narrow-band laser or broadband irradiation produced a variety of pathologic effects. Cell shrinkage and vacuolization were manifest in unfixed preparations observed immediately after

exposure under the phase microscope. Fixed and stained preparations showed increased basophilic staining, pyknosis, and loss of cells evident by "holes" within the irradiated area. Figures 28 and 29 show representative histologic findings from the control and exposed area of a tapetal RPE cell culture. Similarly, Figs 30 through 32 depict cellular changes after exposure in corresponding areas of a pigmented, nontapetal cell culture.

The thresholds of the minimal damage detected histologically by the changes listed above were as follows:

15 mW/cm^2 for 335 to 600 nm
23 mW/cm^2 for 457.9 nm
45 mW/cm^2 for 514.5 nm
800 mW/cm^2 for 590 nm

For comparison, the thresholds of the histologic damage in the macaque retina for a four-hour exposure are given. These were as follows:

2 mW/cm^2 for 457.9 nm
10 mW/cm^2 for 514.5 nm
30 mW/cm^2 for 590 nm

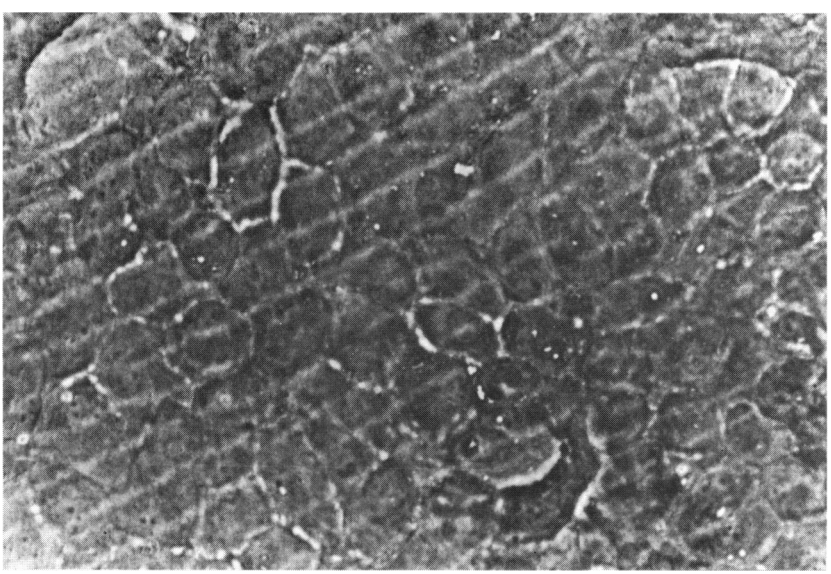


FIGURE 28
Cell culture was derived from tapetal region. Plate was exposed to broadband illumination; however, region photographed was in nonirradiated, control portion of plate. Cells are polygonal, contain no melanin, and show only outline of cell nuclei. Intercellular contact appears to be maintained. Diagonal bands seen in micrograph are caused by striations in plastic culture dish (fixed, stained, phase contrast, ×825).

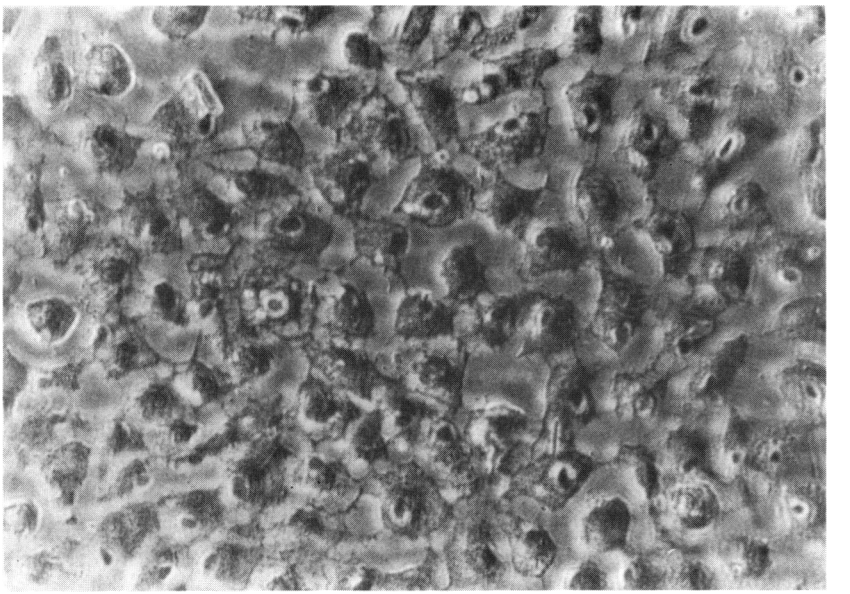


FIGURE 29
Micrograph of same nonpigmented culture shown in Fig 28. However, these cells were in irradiated area. Irradiance on this region was 20 mW/cm^2 with broadband illumination (350 to 600 nm). Cells have shrunk leaving threadlike processes contacting neighboring cells. Presence of vacuoles and pyknosis is evident (fixed, cresyl violet stain, phase contrast, ×825).

There was no difference in sensitivity related to the degree of melanin pigmentation present in the exposed cell.

DISCUSSION

Damage thresholds in the RPE cell culture at 457.9 and 514.5 nm are sufficiently similar to the in vivo threshold to support the conclusion that similar mechanisms may mediate the effects of these wavelengths (450 to 520 nm) in both systems. The irradiance with these wavelengths for producing pathologic change is well below that required to induce thermal effects.

However, there is a great difference between the in vivo and in vitro system in respect to the 590-nm damage. The threshold for this wavelength is 25 times greater in the RPE culture and is sufficiently high to involve thermal processes. Exposing a monolayer of cells on a plastic substrate may significantly alter the heat-diffusing characteristics of the tissue environment in comparison with the in vivo case. The four-hour,

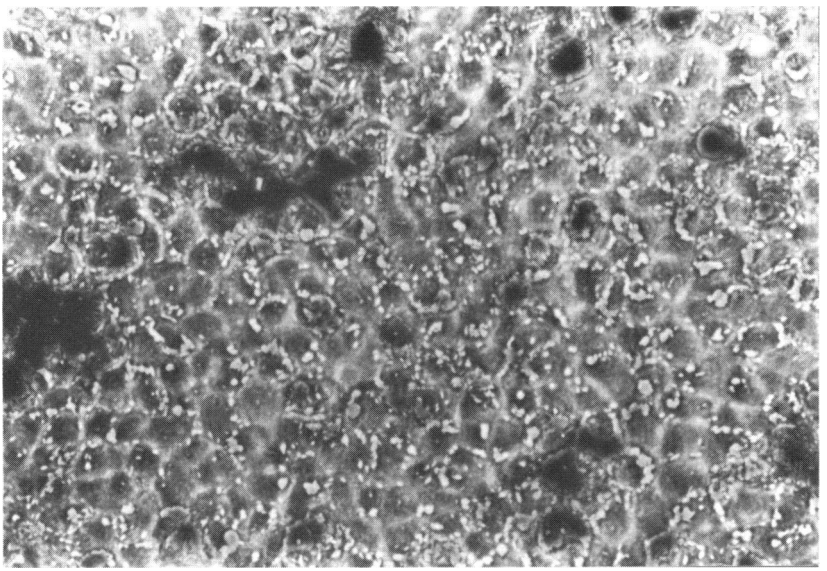


FIGURE 30
Cell culture derived from nontapetal region. Area shown was in nonirradiated, control portion of plate. Cells are polygonal and in apposition to neighboring cells. Moderate degree of pigmentation in most cells is revealed by presence of highly refractile bright flecks corresponding to aggregates of melanin granules. Dense, black structures represent population of nondividing, heavily pigmented cells that may be choroidal melanocytes (fixed, cresyl violet stain, phase contrast, ×400).

590-nm threshold for the retina in vivo is known to be well below the irradiance required to produce thermal damage, but this cannot be said for the in vitro condition; in fact, special measurements will have to be performed to exclude a heat effect.

The important point, however, is the following: Since damage caused by 590 nm in vitro requires 800 mW/cm^2 *v* 30 mW/cm^2 in vivo, this huge difference suggests that the same effect as observed in vivo does not occur in vitro. Thus, the pigment epithelial pathologic changes seen in the retina at 590 nm in vivo may be secondary to the damage to other cells. In this respect, therefore, the mechanism of light damage to the pigment epithelium in the primate would resemble that in the rat (see Introduction). In the rat, the pigment epithelium is not destroyed when light exposure occurs after the photoreceptors have been lost because of a hereditary abnormality.[15] Furthermore, in the rat, the action spectrum of light damage is not compatible with a direct effect of light upon the pigment epithelium.[12] It is assumed, therefore, that the pigment epithe-

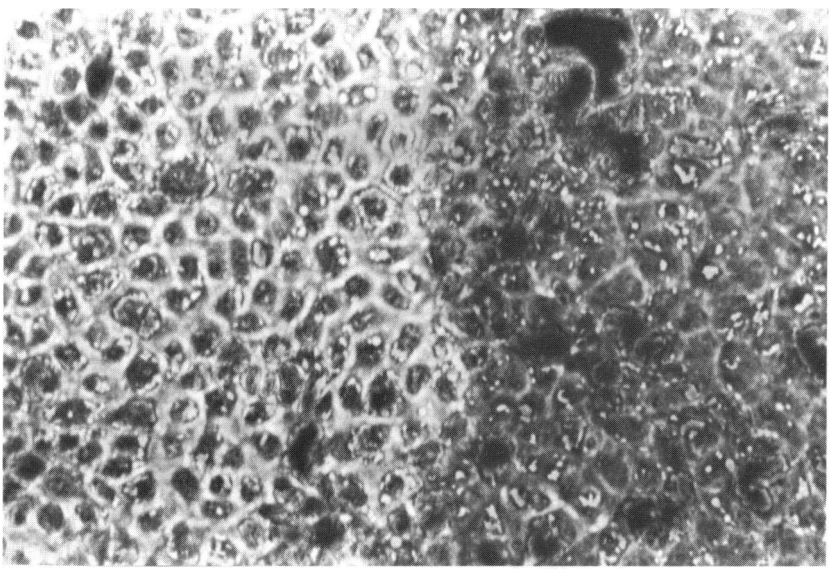


FIGURE 31
Different area of same culture shown in Fig 30. Sharp line demarcates exposed portions (36 mW/cm^2 broadband) on left from control portion on right. Cells in exposed region have shrunk, increasing intercellular space. Cells are pyknotic or show condensation or clumping of nuclear material. Vacuolization of cytoplasm is observed with higher-resolution microscopy (fixed, cresyl violet stain, phase contrast, ×400).

lial pathologic changes are secondary to the action of light on the photoreceptors in the rat model.

In the same way, the effect of 590-nm light on the cultured cells, or the lack of it, suggests that in the primate the pigment epithelium is secondarily involved when 590-nm light produces the damage in vivo. It remains to be studied whether the primary site of action in this case is also the photoreceptor population. It is important to state that the in vivo effect of 590-nm light in the monkey included damage to the pigment epithelium as it did with the other wavelengths. It is important to stress also that the comparison of the in vivo and in vitro conditions excludes the melanin as playing a role in pigment epithelial damage. Contrary to the assumptions by others, melanin may neither enhance the damage nor protect from it.

DISCUSSION

THE THREE PATHWAYS OF LIGHT DAMAGE

Damage of the first kind as described in the rat by Noell[8] has as its prime

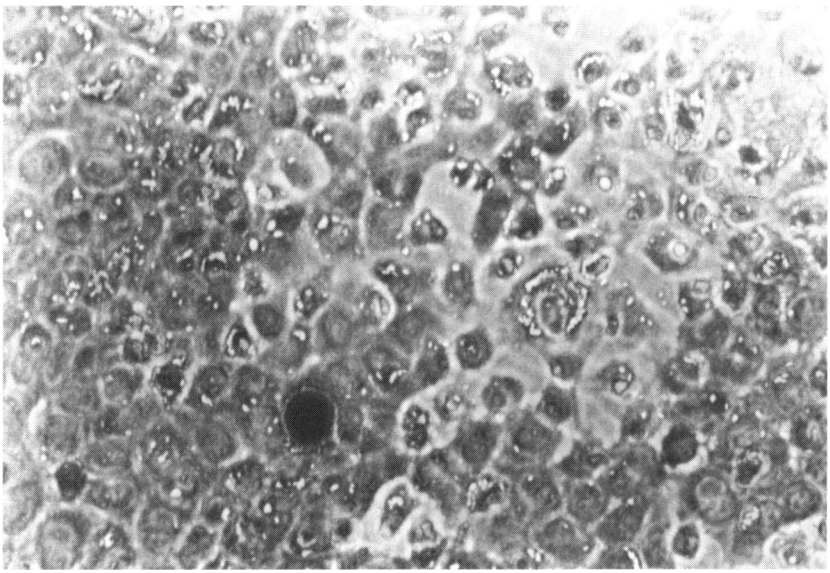


FIGURE 32
Cells from same culture as presented in Figs 30 and 31. Area shown is at border of threshold irradiance (17 mW/cm^2 broadband). Cellular effects range from shrinkage, pyknosis, and vacuolization in upper right corner, to decreased cytoplasmic staining and nuclear halo formation at center right, to minimal changes at lower left (fixed, cresyl violet stain, phase contrast, ×400).

histologic feature the localized loss of the rod cells and of the adjacent pigment epithelium when more than 15% of the rhodopsin is bleached by light applied continuously for two to six days at normal body temperature. He typically notes a loss of all rod nuclei with the preservation of cone nuclei in the area of greatest damage. This type of damage is modulated by body temperature and requires long-term dark adaptation prior to exposure to be fully manifest. The action spectrum of this effect follows grossly the rhodopsin absorption curve, and the bleaching of rhodopsin is most probably the primary change that leads to damage.

Noell[8] described a second kind of damage in the rat, which occurred when the animal was very young or when an adult animal had been kept for weeks in cyclic light prior to exposure. Rhodopsin concentration in these retinas was 15% to 30% lower than in the long-term dark-adapted retinas. The outer segments also tended to be shorter than in the dark group.[42] In this case the pigment epithelium was not lost. Outer segment degeneration was a prominent feature and showed some evidence of recovery. Most probably, rhodopsin is also the mediator of the light

damage of the second kind. Galagos night monkeys and hamsters required more light to produce an effect. And, when produced, it resembled the second kind.

There is only scant evidence of this effect in the primate. It appears that the sensitivity of rhesus rod cells is significantly less than of the rhesus cone cells. Histologic changes similar to those seen by Noell et al[12] in the pigment epithelium and rod cells are only seen in areas where extensive destruction of cone cells and inner layers has occurred. The flash ERG is also much more resistant to reduction in the primate, suggesting that rod function has a high survival potential in the rhesus. Therefore, it seems reasonable to conclude that the pathway to light damage initiated by rhodopsin is not significantly active in the primate under the exposure conditions studied. It follows that the rod cells of the rat (and of the nocturnal Galagos monkey) differ importantly from those of the rhesus monkey.

The second pathway to light damage is demonstrated by Sperling's experiments.[37] Permanent destruction of blue sensitive cones and long-term inactivation of green sensitive cones were observed in primates using exposure to specific wavelengths. In these experiments rod function remained normal. In response to long-term intermittent exposure with intense spectral light, the pathologic finding showed selective loss of cone cells in a mozaic pattern consistent with the distribution of specific cone types. The effect was proportional to the predicted cone density for each type for the central 2°, beyond which the proportion of damaged cones was less than predicted from the distribution of the three cone types. The histologic sections accompanying Sperling's article show moderate to extensive change in the pigment epithelium, particularly prominent in the section 3° from the fovea. Outer segments also appear swollen and disoriented in this section. In the electron microscopic picture the mitochondria are normal, even apparently in one cell that is pyknotic.

With psychophysical tests Sperling was able to show permanent loss of blue sensitivity and reversible loss or green sensitivity in animals exposed to these wavelengths. He was unable to "red blind" the animals with long-wavelength light.

In contrast, the studies reported in this thesis show the combination of two effects. First, the signs of damage found to increase at short wavelengths were evident histologically with each wavelength. In addition, the spectral ERG was reduced across the spectrum for the exposure to every wavelength. Second, a differential effect was evident in the ERG measurements of experiment 2, especially by comparing the effects on

"red" sensitivity after exposure to 457.9 nm (or 514.5 nm) with those after 590-nm exposure.

It is assumed, therefore, that a cone-specific damage is riding on top of a less specific short-wavelength effect and that the cone-specific second pathway to light damage is active in single exposures of one-hour duration. This second pathway is clearly overshadowed, however, by the short-wavelength effect and the pathway and processes upon which this effect depends.

The short-wavelength effect is the main pathway to damage in the rhesus and cynomolgus retinas during single exposures. It affects all layers of the retina. Its action spectrum for clinically observed damage does not follow the action spectrum of rhodopsin or cone pigments. Damage is greater with shorter wavelength 458-nm light than with longer wavelengths. The histologic changes are found throughout the retina including cells of the inner nuclear layer and ganglion cell layers. The degree to which each layer is affected is variable, as are the changes throughout the fundus in any given eye. Pigment epithelial involvement is present in most, but not all, cases. It may be absent even when extensive outer segment damage is evident.

The short-wavelength effect is most easily explained by the work of SantaMaria.[43] He published a series of experiments of direct importance to this one of the three types of pathologic changes. He discovered that isolated bovine retinas, when exposed to light, showed a marked reduction in oxidative respiration, whereas anerobic glycolysis was less affected. He found that liver cells and other tissues not normally exposed to light did not exhibit this inhibition by light. He determined that the inhibition was accelerated in oxygenated media and almost absent when the media was saturated with nitrogen. He determined the effect as a function of time and wavelength. The effect was evident with his intensity of light after ten minutes' exposure. It continued to become stronger for 50-minute exposures and then began leveling off. The spectral action curve peaked between 380 and 500 nm, as close as he could determine with his methods. He notes this effect to be opposite that of roentgenographic irradiation, which affects anerobic glycolysis first. In the rat retina he noted that respiration and glycolysis were affected to the same extent.

Subsequent work on beef heart muscle mitochondria by Ninnemann and co-workers[44,45] is more specific on this subject. She reports inhibition of respiration in beef heart mitochondria by light and the destruction of cytochrome a_3 as the possible primary site of light action. The Sorel absorption band for these cytochromes is at the short-wavelength end of

the visible spectrum and could very well correlate with the histologic and functional effects in this model.

The anatomic description presented in this thesis shows one group of organelles to be by far the most affected by light exposure: the mitochondria. Also, specific mitochondria of the "fat" type found in cones are differentially susceptible. The mitochondria located in the inner layers of the retina are in some instances more affected than the packed mitochondria in the inner segments. One might relate this to possibly a lower oxygen tension at the receptor layer than near the capillaries of the inner layers. The hypothesis that the initial damage is in the mitochondria is especially attractive, since the mitochondria contain potentially photodynamic compounds such as cytochromes and flavoproteins, some of which happen to have an absorption band in the 390- to 440-nm region, the short-wavelength region of light damage.

I propose that the short-wavelength effect as described in my thesis (and the effect studied by Ham and Mueller[10]) has the following mechanism: Light passing through the retina is absorbed by all mitochondria. Wavelengths between 390 and 440 nm are specifically absorbed by some components, possibly a cytochrome. This destroys a specific reaction in the oxidative cycle. It causes the cell to drastically decrease its function but not die, because energy supplied by glycolysis is sufficient to maintain the integrity of the plasma membrane of the cell. Glycogen builds up; function continues at a reduced rate, and returns toward normal only in those cells that have retained sufficient functional mitochondria to repair the damage and whose nuclei provide information to rebuild mitochondria. The continuing production of distorted outer segments could be supported by a reduced energy source. The distortion could be secondary to either mechanical distortion of the cell or, more likely, abnormal synthesis and abnormal membrane assembly. The abnormal glycogen accumulation might be secondary to a decrease in the rate of oxygen consumption.

Given this mechanism, it would appear that destruction of a majority of mitochondrial activity in any cell is necessary before the cell succumbs to damage. The mitochondrion contains sufficient genetic information to do all but reproduce itself. Repeated exposures would be expected to be additive to some extent, but this addition would be decreased by any repair that could occur between exposures. The fact that mitochondria cannot reproduce themselves but must wait for nuclear input puts a total repair on a long time scale, especially since the respiration of the cell is compromised. The meaning of this might be that cumulative short-wavelength light effects may be an important factor in the retinal pathologic

changes of advanced age. At the other end of the age spectrum, we must consider that the short-wavelength effect may be enhanced by high oxygen concentration and that the infant's retina may be endangered if both phototherapy and supplemental oxygen are administered.

In discussing the type and location of histologic damage seen in the monkey retina, one must address what is primary and what is secondary. Work with functional measures had led us to evaluate the histologic changes, especially at a period one to three months after exposure. This long after exposure, scattered damage throughout all layers of the retina is evident. Individual hyperchromaticity can be seen most prominently in receptor cell bodies and processes. The inner and outer segments are distorted in their form. The pigment epithelium also presents pathologic changes. However, function as measured by the ERG returns to normal in two to four weeks in many instances. Several questions exist: (1) Do these distorted inner and outer segments function normally or nearly so? (2) Do the hyperchromatic cells become this way immediately, or are cells still dying three or more months following the insult? There are gaps found in the pattern of receptors to suggest that cells are missing. When were they lysed and removed? Appearances suggest that some may just fade away slowly while other cells remain fossilized. Or, are these darkened cells with condensed cytoplasm and nuclei still metabolically surviving and even functional?

For these reasons, only an arbitrary definition of primary and secondary can be given. Primary refers to a direct, immediate effect of light on the cell in question, from which it does not recover completely. Secondary includes effects in the same or different cells, which may develop with time after exposure. The data presented 1 hour, 24 hours, and 7 days after exposure are not significantly different from those studied at longer survival intervals up to 5 months. The comparison must be made with a clear understanding of the variability in degree of damage from eye to eye and from area to area within each eye. Already at one hour, hyperchromatic nuclei are present in the outer nuclear layer and condensed cytoplasm. Cells in the inner nuclear layer and ganglion cells are also disintegrating at this time. The pattern seen later is the same except for (1) spaces left in the cellular pattern, (2) pale staining cone nuclei, and (3) enlarged and disorganized processes in the outer plexiform layer. All these are most obvious at later times. It is concluded, therefore, that the main histologic changes after a single exposure result from the direct, immediate effect of the damaging light. These are most probably "primary" effects.

Because the retina is such a complex organ, it is likely that the individual cell's sensitivities will change depending upon the environment and

level of activity. Therefore, studying each cell type separately or even in binary combination will only give a hint as to their susceptibility in the functioning organ. Nevertheless, such a systematic approach is warranted because of the importance of the light effect and the reputed possibility of potentiation of some hereditary retinal degenerations or retinal toxicity from other agents or both. The studies with the cultured pigment epithelium are a start of this research.

There are many unknowns in the relationships between exposure and effect for which new data are required: (1) As emphasized, there is a large difference in susceptibility across species. (2) There is a difference in the most susceptible cell type not only across species but also depending upon the intensity-time-wavelength parameters of the damaging stimulus in the same species. (3) In some models basal body (retinal) temperatures have a profound effect, while in others they do not. (4) The anatomic damage in individual cells appears almost all or none. This digital response may have as its basis the efficient repair mechanisms that are continually active. Some cells die, some live, very few are in between. (5) While each animal model shows a most sensitive area of retina, it is not the same area in the different animals. The rabbit is the most striking case, where the damage occurs in an oblong area with a vertical-horizontal disparity of more than 1:4. Certainly, this is well beyond any differential in retinal illumination. (6) The area of greatest susceptibility appears to reflect the area of the greatest number of receptors, the central area in primates and the visual streak in rabbits. (7) There is little effect of choroidal and pigment epithelial melanin concentration upon the susceptibility of the retina in rabbits. In variegated fundi the strip of damaged area shows no discontinuity or change in dimension crossing over the sharp borders of change in pigmentation. (8) There must be influence from factors such as metabolic demand, nutritional supply, synthetic activities, plasma membrane properties, genetic makeup, and systemic or ocular abnormalities. (9) The area of retina most susceptible to one type of light damage is also that area most suceptible to chloroquine in the primate. The susceptibility therefore does not necessarily follow the patterns of greatest illumination. The retina can be made even more susceptible by toxins or deficiencies, be they externally or internally (geneticall-y) modulated. (10) Leaving a frog in darkness can also lead to retinal degeneration. This might suggest that any sudden change in the biologic rhythm can cause a deleterious effect on this highly sensitive tissue, the retina.

These and many other questions are open to future research. I am confident that most can be answered within the foreseeable future and

that the time is not far off to apply the experimental work to the patient. I hope that the work described in this thesis will make it easier to reach this goal.

SUMMARY

This thesis presents information important in present-day research and understanding of damage by light to the retina. It presents a description of three distinct pathophysiologic mechanisms of light damage that combine in various proportions in different animal models. The pathologic changes in each species depend upon a unique mixture of the three mechanisms. The proportion of damage represented by each mechanism is dependent upon the characteristics of the species and the physical parameters of intensity, wavelength, and duration of the damaging light.

The first mechanism is visual pigment, rhodopsin-specific. It is seen in the rat and some other nocturnal animals with rod cells that are several orders of magnitude more sensitive to damage than primate rods, and more sensitive than their own cones. This mechanism is almost nonexistent in the primate. It is as though the rods of rhesus and cynomolgus monkeys are for some unknown reason specifically resistant to light damage and are only affected when all the cells around them have already been severely damaged.

The second mechanism is cone pigment-specific and occurs in the most pure form in primates when long-term, repeated low-intensity spectral exposures cause damage. It is also seen in combination with the third mechanism, short-wavelength light effect, as shown in this thesis. Long-term functional loss of specific cone populations (red-sensitive, green-sensitive) following spectral exposure is demonstrated by ERG chromatic sensitivity. This mechanism is overshadowed in the primate by the short-wavelength light damage, which is considerably less specific.

The third mechanism, which is responsible for light damage in the primate in single exposures, has an action spectrum peaking in the short-wavelength (blue) end of the visual spectrum. It leads to damage in all layers of the retina, from the pigment epithelium through the nerve fiber layer. For this effect, the thesis presents a definitive, testable hypothesis as to its mechanism. The hypothesis is that the effect is caused by a direct action of light on the mitochondria in the different retinal layers, which inactivates the respirating enzymes, other effects being secondary to or additive with this major mechanism.

Three experiments are presented as well as a detailed account of work by the author over the last ten years. The first experiment on more than

80 monkeys establishes the action spectrum of light damage in the primate retina for one- to four-hour single exposures. Its results provide an all-inclusive description of the histopathologic features of this effect and how they vary with the physical exposure parameters. The progress of these changes is followed for periods ranging from one hour to five months after exposure. Measurements include a clinical (ophthalmoscopic and fluorescein angiographic) description and grading of the damage and flash ERG recordings. The clinical and histologic descriptions depict a great variability in the severity of damage as well as of its patchy location in specific layers and retinal regions. The general trend shows the damage to be most concentrated in a concentric ring between 3° and 5° from the fovea and to be located histologically throughout all layers of the retina and pigment epithelium.

In the second experiment, the chromatic bar pattern ERG was utilized to determine chromatic sensitivity before and after narrow-band light exposures. It demonstrates specific cone-type susceptibility overshadowed by the short-wavelength (blue) light effect.

The third experiment exposes pigmented and nonpigmented epithelial cells in culture. There is no difference in damage threshold between the pigmented and nonpigmented cells. The striking difference is between the action spectrum in vivo and in vitro. The damage thresholds at 457.9 and 514.5 nm are relatively similar, but for 590 nm the sensitivity is 25 times less in culture (800 mW/cm^2). The pigment epithelium's participation in the in vivo 590-nm damage (30 mW/cm^2) appears to be secondary to effects of the light on adjacent tissues.

ACKNOWLEDGMENTS

I should like to acknowledge the contributions and participation of the following individuals in the experimental studies: Steve Crockett, PhD, who has been intimately involved in all of these projects for over ten years. Glenna Currier, MS, who is responsible for the beauty and accuracy of the histological preparations. Robert Rosenberg, PhD, who carried out experiment 2 as part of his PhD thesis under my guidance as major professor.

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